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EFFECT OF ENVIRONMENTAL AND CULTURAL FACTORS ON THE DWARF DISEASE OF ALFALFA [1]

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INTRODUCTION

In an earlier article (*14*)[2] the writer has described briefly, under the name "dwarf", a previously unreported disease of alfalfa (*Medicago sativa* L.), the cause of which is unknown. The fact that the dwarf disease is limited to the southern half of the State of California suggests the possibility that there may be some climatic conditions which tend to limit its distribution or some cultural factors which affect its severity. Temperature, soil moisture, soil fertility, and cutting in an immature stage are factors that appear to be worthy of consideration. It has been suggested by some that the disease might be caused by one or more of these factors.

It is the object of this paper to report experiments and observations designed to show what effect, if any, certain cultural and environmental factors may have on the development of the dwarf disease and on the longevity of alfalfa stands in southern California.

METHODS AND MATERIAL

For these investigations, 16 plots, each 1 by 4 rods in area, were laid out in a 1-year-old alfalfa field on the experimental farm of the University of California at Riverside, Calif. Although this field had been dry-farmed to cereal crops for a number of years, it had never grown alfalfa. The alfalfa used was the so-called Chilean variety, which is most commonly grown in southern California. When the plots were laid out, on July 1, 1929, the stand was in excellent condition in regard to both number and growth of plants. Since the ground was only fairly level, the distribution of water had never been quite even; however, neither the stand nor the vigor of the plants was in any way impaired. This semidrought condition was easily remedied by a revised system of irrigation instituted for the plots, which were so arranged that each could be irrigated separately whenever desired. Figure 1 shows the arrangement of the plots in two series, of eight plots each, separated by an irrigation ditch and bordered on the east by another irrigation ditch. In each series border checks, each 4 feet wide, separated the plots into four groups of two each; the individual plots in each group were separated by an embankment. The two plots of each group were always irrigated alike. Since the "run" was only 4 rods long, it was possible to cover

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[2] Reference is made by number (italic) to Literature Cited, p. 367.

any plot quickly and evenly. Usually the water was allowed to remain on the plot for 4 hours after the ground had been entirely covered. By means of a soil auger it was found that this was sufficient time to soak the soil to a depth of 3 feet or more. Plots 1 to 4 and 9 to 12, inclusive, were irrigated once a month, and the remaining plots twice a month. However, plots 1, 2, 5, 6, 11, 12, 15, and 16 were allowed to go without irrigation during the seed-producing period each summer (about 3½ months).

EFFECT OF SOIL MOISTURE

Early in these investigations field observations indicated that soil moisture exerted a very decided influence on the development of the

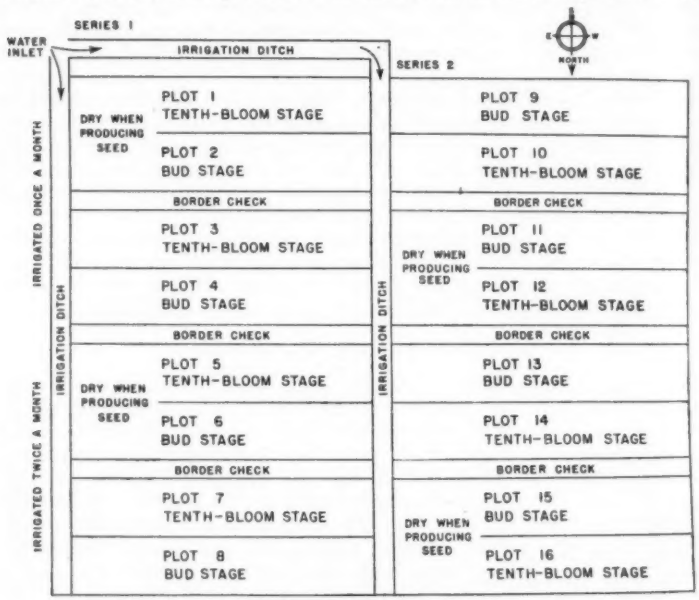

FIGURE 1.—Diagram showing arrangement of plots used in experiments.

dwarf disease and consequently on the length of life of the alfalfa stands. It was noted that the alfalfa stands which died out first were those that received the most irrigation water or those in low spots where the water collected. Often a stand was very good in the high spots of a field, where water reached only with difficulty or sometimes not at all, but elsewhere it was quite thin. Likewise, in certain small valleys where growers do not irrigate their alfalfa but depend entirely on winter rainfall for their moisture supply, dwarf is practically nonexistent and the stands continue for many years.

Having been convinced from field observations that soil moisture is a factor in the development of dwarf, the writer sought confirmatory experimental evidence based on the stands in plots given various irrigation treatments.

The stand was determined by counting the plants in three 3-foot-square quadrats in each plot, one at the center and the others about

a third of the distance from each end. These quadrats were permanently located at the beginning of the experiment by driving a stake at each corner. Although the quadrats for the most part were fairly representative of the conditions, in a few cases, where the plants on one side or corner of the plots died more rapidly than those in the quadrat, the count taken was too high. This was especially true of plots 1 and 9, in which the plants died more rapidly along the south side than elsewhere; in fact by the end of the third season the stand in these plots was so poor that the yield figures were considered valueless.

TABLE 1.—*Effect of irrigation treatments on alfalfa stands from September 1929 to October 1931*

[Data represent averages from three 3-foot-square quadrats in each plot]

Number of irrigation treatments per month	Plot no.	Average number of plants					Percentage of plants (October 1931)—	
		September 1929	April 1930	September 1930	March 1931	October 1931	Dead	Living
1	[a] [b] 1	45	49	35	24	14	69	31
	[c] [b] 2	76	68	56	44	35	54	46
	[a] 3	51	55	47	44	40	22	78
	[c] 4	54	53	40	38	13	76	24
	[c] 9	69	63	42	46	10	86	14
	[a] 10	62	58	47	48	41	34	66
	[c] [b] 11	68	58	41	46	40	41	59
	[a] [b] 12	57	52	49	40	39	32	68
2	[a] [b] 5	67	69	52	52	53	21	79
	[c] [b] 6	68	62	47	48	45	34	66
	[a] 7	73	57	50	48	12	84	16
	[c] 8	60	45	37	40	0	100	0
	[c] 13	84	58	54	52	6	93	7
	[a] 14	80	54	48	44	14	83	17
	[c] [b] 15	89	59	65	58	55	38	62
	[a] [b] 16	76	55	55	50	43	43	57

[a] Plants cut in tenth-bloom stage.
[b] Plants dry when producing seed.
[c] Plants cut in bud stage.

The number of plants in the different plots, as represented by the average of the three quadrats, is given in table 1. The difficulty involved in making accurate counts of alfalfa plants is appreciated by those who have attempted it. Sometimes two or more plants are growing so close together that it is impossible to tell how many are present without removing considerable soil from about the crowns. In the present instance the soil was not removed, but every quadrat was counted by two men, each counting the entire quadrat, and the average of the two counts was used. In case the counts differed by more than a few plants, a recount was made by one or both men. Counts were made in the spring and in the autumn of each year. The results of these counts are presented in table 1. The number of plants per square foot in September 1929 ranged from 5 in plot 1 to 9⅝ in plot 15. For the most part the figures show that there was a gradual thinning of the stand, varying in rapidity in different plots, so that by October 1931 some plots were still quite good while others had few or no plants left. At that time the stands in plots 1, 3, 4, 7, 8, 9, 13, and 14 were very thin, appearing even thinner than the percentage of living plants recorded in the table would indicate.

As is often the case, the stands did not always thin out uniformly; frequently there were more than the average number of plants in a quadrat, thus making the average higher than it would have been had it been possible to count all the plants in the plot. For example, table 1 shows 78 percent of a stand left in plot 3 in October 1931, while estimates made at the same time indicated that there was about 20 percent of a stand at one end and 70 percent at the other end of the plot, or an average of about 45 percent for the plot as a whole. On the other hand, certain quadrats thinned out more than the average of the areas they represented.

In view of the fact that a gradual decrease in the number of plants in each plot took place during the 3 years the experiment was in progress, it becomes of interest to consider the reasons for the unequal rate of thinning. As already stated, some plots (1 to 4 and 9 to 12, inclusive) were irrigated once a month during the growing season, whereas the others were irrigated twice a month, and plots 1 and 9 thinned out more rapidly along the south side than did the others; but since other factors, especially a difference in soil type, were involved in plots 1 and 9, these two plots are not strictly comparable with the rest and are therefore omitted from the present discussion. The remaining figures show an average loss of 43 percent for the plots irrigated once a month and 62 percent for those irrigated twice a month. Another factor is involved, however, the fact that plots 2, 5, 6, 11, 12, 15, and 16 were not irrigated during the seed-growing period. For the plots similarly irrigated the following averages were obtained: The average percentage of loss in stand for the plots irrigated once and for those irrigated twice a month throughout the season was 44 and 90, respectively, as compared with 42⅓ and 34, respectively, for the plots similarly treated but left unirrigated during the seed-producing period. These figures show that there was a decidedly more rapid thinning of the stand in the plots irrigated twice a month, which, however, was counteracted when the stands were kept dry during the seed-producing season (figs. 2, 3, and 4). The difference in the rate of thinning of the two groups of plots that were allowed to go to seed is probably not significant.

Figures 2, 3, and 4 show the condition of certain of the plots in October 1931, when the experiment was discontinued. There seems to be a discrepancy between the data for plot 3 given in table 1 (78 percent of plants in quadrats living in October 1931) and the appearance of the plot in figure 2. This is probably due, at least in part, to the fact that the number of plants in the quadrats was not truly representative of the condition of the plot as a whole. Plot 3 was one of the best plots throughout the experiment and yielded well (table 3). However, during the late summer and autumn of 1931 the plants died out quite rapidly.

The conclusion seems justified that under the conditions of this experiment the rate of thinning of the alfalfa stand was greatly increased, in fact was practically doubled, by doubling the number of irrigations, provided the stands were not allowed to go without irrigation during the summer months. This experiment, therefore, seems to confirm the field observations indicating that soil moisture is an important factor in the longevity of the stands of alfalfa in southern California.

FIGURE 2.—Several alfalfa plots, showing the effect of different cultural treatments. In foreground, plots 3 and 4, irrigated once a month and cut regularly for hay; vegetation mostly weeds. At left, part of plots 1 and 2; at right, part of plots 5 and 6. In background, several plots of second series (9 to 16, inclusive); the two men shown are standing on bank of irrigation ditch separating the two series of plots. Just back of plots 3 and 4 are plots 11 and 12. Plots 1, 2, 5, 6, 11, 12, 15, and 16 were left unirrigated each summer during growth of seed crop. To right of plots 11 and 12, plots 13 and 14; still farther to right, plots 15 and 16. (For details of arrangement and for treatment given each plot, see figure 1.) Photographed in October 1931.

FIGURE 3.—Closer view of plots 5 and 6, showing condition of stand in plots irrigated twice a month except when growing seed. Photographed in October 1931.

FIGURE 4.—Closer view of plots 13 and 14, showing condition of stand in plots irrigated twice a month and cut regularly for hay. Vegetation mostly weeds; only a few scattered alfalfa plants. Compare with stand of alfalfa in plots 11 and 12 (left) and 15 and 16 (right), where seed was produced each summer. Photographed in October 1931.

EFFECT OF FREQUENCY OF CUTTING

In southern California it is a common practice to cut alfalfa before the blossoms open, in order to meet the market demand for such hay to supply cattle and rabbit feed. This practice is believed by some to be responsible for the rapid thinning of the stand. Several investigators have shown (*3*, *8*) that cutting alfalfa when too immature shortens the life of the stand somewhat, at least under certain conditions. An experiment was therefore designed to ascertain whether cutting in the bud stage, as compared with cutting in the tenth-bloom stage, makes any appreciable difference in the longevity of the stand. This part of the experiment was undertaken in conjunction with the study of soil moisture recorded herein, and the discussion must be based on the figures given in table 1. In this experiment, as shown in figure 1, every other plot was cut in the tenth-bloom stage and the alternate ones in the bud stage, except when the stands were allowed to go to seed. In the first series (fig. 1) the odd-numbered plots were cut in the tenth-bloom stage and the even-numbered plots were cut in the bud stage; in the second series the order was reversed.

As already stated, plots 1 and 9 are not strictly comparable with the rest; hence they and their companion plots (2 and 10) are omitted from this discussion. Since the plots run in groups of two, perhaps the best way to consider them is in that order. For example (table 2), plot 3, cut in the tenth-bloom stage, and plot 4, cut in the bud stage, have lost 22 and 76 percent, respectively, of their original stands. In like manner, plots 5, 7, 12, 14, and 16 may be compared with their companion plots, 6, 8, 11, 13, and 15, respectively. Such a comparison indicates that for the most part there is a slightly greater loss of plants in plots cut in the bud stage than in the other plots. The reverse is true for plots 16 and 15; however, the small difference is probably not significant. It is also noticeable that (omitting plots 16 and 15) the differences, though always in the same direction, are not large, except that between plots 3 and 4. The wide difference between these two plots is probably due to the fact that the figure for plot 3 is not representative of the plot as a whole, as previously explained. A further study of table 1 shows that the plots which thinned out so completely did so largely during the summer of 1931. Further light on just when this rapid dying of the plants occurred is given by the yield data in table 3, which show that all the plots, except plot 1, yielded fairly well for the first three cuttings. A falling off in yield is noticeable in plots 4, 7, and 9, in the fourth cutting, and a decided reduction is evident in plots 4, 7, 8, 9, 10, 13, and 14, in the fifth cutting, which was made between June 19 and July 29. It seems evident from these data, as well as from many field observations, that the rapid reduction in stand starts about the middle of June and, if conditions remain favorable for the development of the disease, continues until the stand is nearly or entirely depleted. Doubtless the most rapid dying of the plants takes place during July and August.

Just why the yields of plots 3 and 10 should have increased so greatly at the fourth cutting is not evident from the data at hand. These plots, as well as several of the others, showed more or less decrease in yield at the third cutting, followed by an increase at the

fourth cutting. It seems to the writer most probable that soil moisture was at least one of the major factors involved in these fluctuations. This is suggested by the fact that the greater variations occurred in the plots irrigated only once each month. Occasionally the water was not available when needed, so that the plots irrigated only once a month suffered somewhat from drought.

TABLE 2.—*Effect of cutting plants at different stages of maturity on percentage of loss in stand*

Stage when cut	Plot no.	Percentage of plants dead	Stage when cut	Plot no.	Percentage of plants dead
Tenth bloom	3	22	Bud	4	76
	5	21		6	34
	7	84		8	100
	12	32		11	41
	14	83		13	93
	16	43		15	38

The total yields for the year are not of much value, since they are influenced by two inseparable factors, namely, thinning of the stands and weakening of the plants, the latter due, no doubt, to the reduction of root reserves as described by Graber and his coworkers (*3*). This weakening, which was evident as a shortening of top growth, was quite noticeable at times, especially during the latter part of the season, even before the stand started to thin so rapidly. However,

TABLE 3.—*Yield (in pounds) of weed-free hay for each cutting and the total yield for 1931*

Plot no.	Pounds of weed-free hay from indicated cutting							
	1	2	3	4	5	6	7	Total
1	3.3	[a]	[a]	[b]	[b]	[c] 52.57	27.47	83.34
2	23.8	50.46	58.17	[b]	[b]	[c] 78.47	66.66	277.56
3	32.1	86.00	52.26	96.96	85.00	52.52	[d]	404.84
4	21.8	38.25	43.53	34.84	16.36	0	3.73	158.51
5	39.7	104.19	93.93	[b]	[b]	[c] 75.15	62.71	375.68
6	36.1	75.48	60.05	[b]	[b]	[c] 70.19	48.20	290.02
7	31.5	93.12	71.08	21.81	1.99	0	0	219.50
8	20.6	34.77	34.10	40.29	3.00	0	0	132.76
9	14.2	51.07	51.26	39.23	17.59	0	0	173.35
10	27.1	91.64	58.01	86.10	43.84	55.35	[d]	362.04
11	18.3	64.94	61.94	[b]	[b]	[c] 86.88	63.67	295.73
12	31.7	92.37	66.35	[b]	[b]	[c] 97.53	67.34	355.29
13	23.7	36.54	49.98	54.28	2.50	0	0	167.00
14	28.3	83.70	66.63	75.41	38.08	0	0	292.12
15	33.3	69.37	66.75	[b]	[b]	[c] 112.67	62.18	344.27
16	44.1	115.46	90.04	[b]	[b]	[c] 120.74	63.48	433.82

[a] Not weighed, owing to large quantity of weeds.
[b] Plots allowed to go to seed.
[c] Weight of straw from seed crop.
[d] There was no seventh tenth-bloom cutting.

table 4, which gives the total yields by plots and, like table 2, is so arranged that the adjacent plots of each group can be compared readily, shows that in every case the plots cut in the tenth-bloom stage yielded considerably more hay than the adjacent plots cut in the bud stage. The total yield for all the plots cut in the tenth-bloom stage is approximately one third greater than that for the plots cut in the bud stage.

TABLE 4.—*Total yields (pounds) of weed-free hay in 1931 from plants cut in the tenth-bloom stage and from those cut in the bud stage*

Stage when cut	Plot no.	Yield	Stage when cut	Plot no.	Yield
Tenth bloom	3	404.84	Bud	4	158.51
	5	375.68		6	290.02
	7	219.50		8	132.76
	12	355.29		11	295.73
	14	292.12		13	167.00
	16	433.82		15	344.27
Total		2,081.25	Total		1,388.29

DWARF AS A FACTOR IN RAPID THINNING OF STANDS

It has been pointed out how soil moisture and possibly to a certain extent frequency of cutting may tend to shorten the life of the alfalfa stands. The chief object of the present experiment, however, was to determine, if possible, what effect these factors may have on the rate of spread and on the severity of dwarf. The percentage of diseased plants in each plot was determined in the spring and autumn of each year of the experiment; the figures thus obtained are given in table 5. For the first three counts 25 plants, irrespective of whether they were diseased or healthy, were dug from each corner of the plot. It was then decided that a more representative sample could be obtained by digging all the plants in two strips, each 1 foot wide, extending across the plots and located about one third of the distance from either end of the plots. Since the strips in which the counts were made in November 1931 were adjacent to those used in February 1931 they were comparable in every way. The number of plants obtained from each plot by this method ranged from 50 to 125, depending upon the thickness of the stand. Every root was peeled and examined, so that the early stages as well as the late stages of the disease were observed.

TABLE 5.—*Percentage of diseased alfalfa plants in each of the 16 plots on various dates*

Plot no.	Percentage of diseased alfalfa plants on indicated date				
	October 1929	April 1930	September 1930	February 1931	November 1931
1	53.1	36.2	36.0	44.6	(a)
2	21.9	10.3	33.0	16.4	17.7
3	13.3	10.5	20.0	12.4	63.0
4	4.0	10.9	30.0	22.1	26.0
5	0	5.1	4.0	3.0	37.0
6	4.4	4.8	24.0	11.7	36.0
7	8.5	16.2	64.0	65.3	(a)
8	0	16.0	50.0	77.5	(a)
9	6.2	17.0	42.0	52.3	(a)
10	0	6.9	15.0	16.3	31.5
11	2.5	1.0	6.0	13.4	25.5
12	1.2	1.0	7.0	6.8	30.0
13	5.3	3.0	31.0	50.1	41.7
14	5.3	6.0	19.0	36.6	40.0
15	2.6	2.9	3.0	4.1	10.4
16	0	1.0	7.0	12.7	25.0

[a] Stand so depleted that there were not enough plants left to make any reliable counts.

The data in table 5, column 2, show the amount of disease present in October 1929, when the stand was a little less than 2 years old. It will be observed that there was considerable disease in plots 1 to 3. The presence of the disease can be explained by the fact that these plots were nearest to the source of water and had received considerably more water than the others at each irrigation before the experiment was started. It is interesting to note that as high as 53 percent of diseased plants were present in plot 1 in the second year of the stand on soil which had never before grown alfalfa and which was several miles from any other field of growing alfalfa. It should be stated, however, that another alfalfa field, about half a mile away, had been plowed in the autumn after the plots under discussion were seeded. Nevertheless, it is difficult to see how this could have been of much importance as a factor, for the irrigation water did not come in contact with the old stand on its way to the new one and the prevailing winds blew in another direction.

Except in plots 1 to 3, there was comparatively little disease in October 1929. The following spring more diseased plants were found; yet, with the exception of plot 9, they were confined largely to the east end of the series on the east, including plots 1 to 8. From that time the disease spread so rapidly that by November 1931 some plots had only an occasional plant left.

From the data in table 5 it may be determined whether a correlation existed between either soil moisture or frequency of cutting and the development of the disease. It must be borne in mind that plots 1 to 4 and 9 to 12, inclusive, were watered once a month and the others twice, and that plots 1, 2, 5, 6, 11, 12, 15, and 16 were without irrigation during the seed-growing period each summer. As noted previously, plot 1 had a high percentage of diseased plants at the beginning (table 5); and, as indicated by the yield data in table 3, the stand died out rapidly. Although the northwest corner remained fairly good throughout, for the most part plot 1 did not show any noticeable response to the treatments given it; it seems best, therefore, to omit plot 1 from the comparisons that follow. It should also be pointed out that some of the figures for November 1931 may be misleading. For example, plot 4 seems to have had less disease in November than plot 3, although the reverse had been true previously. This difference is due to the fact that not enough plants were left in plot 4 in November 1931 to make a representative sample; hence this figure had little or no significance. A comparison of each group of companion plots (3 and 4, 5 and 6, etc.) shows few very striking differences. The outstanding difference apparent between plots 9 and 10 can be explained by the fact that in plot 9, as in plot 1, the plants died more rapidly from the start than in the plot adjacent to it. In many cases the plots cut in the bud stage had a higher percentage of dwarf than the corresponding plots cut in the tenth-bloom stage. There were several instances, however, where the disease was equally prevalent in both plots or where the plot cut in the tenth-bloom stage was the more seriously affected. In February 1931 plots 4, 6, 8, 11, and 13, which were cut in the bud stage, had higher percentages of disease than did the adjacent plots (3, 5, 7, 12, and 14). The reverse is true of plots 15 and 16. In September 1930 plot 7 had more disease than plot 8, and plot 16 had more than plot 15. These

figures fail to give convincing proof that the disease is very greatly influenced by the stage of maturity of the plants when cut; hence it is difficult to see how cutting in the bud stage could be responsible for the dwarf disease.

The correlation between soil moisture and the prevalence of dwarf may be determined from the counts made in February 1931. These counts seem to be as suitable as any, if not the most reliable of all, since the samples were taken in two uniform strips across the plots at a time when the disease was very prevalent but when the stands, except that of plot 1, were not too thin. For the plots (except 1 and 9) of the series irrigated once a month, the average percentage of disease was 14.6; and for those irrigated twice a month, 32.6, a difference of 18 percent. This difference is even greater when the plots allowed to go to seed are excluded, the averages being 16.9 and 57.4 percent for the plots irrigated once and twice a month, respectively, or a difference of 40.5 percent. If those plots which had exactly the same treatment are compared, the respective percentages of diseased plants will be found to be as follows: Plots 2 and 11, 16.4 and 13.4 percent; plots 3 and 10, 12.4 and 16.3 percent; plots 5 and 16, 3.0 and 12.7 percent; plots 6 and 15, 11.7 and 4.1 percent; plots 7 and 14, 65.3 and 36.6 percent; and plots 8 and 13, 77.5 and 50.1 percent. These figures show a fairly consistent amount of disease in the duplicate plots. They also emphasize the great increase caused by the added amount of water when the applications were made throughout the growing season, and show the effect on the wet series of the summer drought, which seemingly had no effect on the dry series.

Although only one experiment is involved, the results with respect to the effect of soil moisture on dwarf are so striking and consistent and check so closely with field observations that the following conclusions seem to be justified: (1) That the alfalfa dwarf disease is greatly influenced by soil moisture; (2) that high soil moisture during the summer months is conducive to the spread of dwarf; and (3) that the reverse is true of low soil moisture. The exact degree of soil moisture conducive to the increase or decrease of dwarf has not been determined. Seed production, with the attendant dry period, has a tendency to retard the spread of the dwarf disease, especially if the soil moisture has been high at other times.

The question now arises as to whether soil moisture caused the decrease in stand through its effect on the development of dwarf; that is, whether dwarf was the sole or at least the major cause of the premature thinning of the stands in these alfalfa plots. Probably this question can best be decided by comparing tables 1 and 5. The percentage of plants lost in plot 3, as given in table 1, was rather low; the percentage of disease in the same plot, as given in table 5, was comparatively low until November 1931. Nearly all the plants indicated as diseased in November 1931, however, had an early stage of dwarf; hence they would not have died until the following year. The correlation does not seem to hold for plot 4, in which the percentage of plants lost was high (table 1) and the percentage of disease was comparatively low (table 5). In most of the other plots there seems to be a fairly close correlation between the plants lost and the amount of dwarf. Owing to errors in sampling, which may be fairly high in some cases, a considerable variation must be expected. Furthermore, it seems probable that some of the plants that died

were not affected by dwarf. In fact, it is known that some plants were so severely affected with a crown rot that no tops were formed, although the roots still appeared to be healthy. On the other hand, the roots of a considerable number of plants made little growth. Although such plants were hardly ever affected with dwarf, many no doubt eventually died. In general, however, it is thought that the majority of the plants that were lost died from dwarf.

EFFECT OF TEMPERATURE

No definite experiments have been conducted to determine the influence of temperature on dwarf. Field observations, however, tend to refute the supposition that temperature is an important factor in the spread or the severity of the disease. Dwarf has been found to be as severe in places near the coast, where the temperatures are comparatively low, as in the interior valleys, where they are considerably higher. For example, temperatures recorded during June, July, August, and September, for 42 years in Los Angeles and for 23 years in Riverside (*12*), show that the mean and the mean maximum temperatures for Los Angeles were 5° and 10° F. lower, respectively, than those for Riverside. With respect to both the mean and the mean maximum temperatures more extreme conditions doubtless exist in many alfalfa fields, since alfalfa dwarf is found in places much nearer the ocean than Los Angeles, as well as in places where the mean temperatures are higher than those officially recorded for Riverside. That seasonal temperatures do affect the development of dwarf in the plant is indicated by the fact that the diseased wood in an affected plant is commonly overgrown during the winter months. As early as the 1st of December a thin white layer of new wood covering the yellow diseased area may be found in many plants. New wood continues to develop during the winter, so that by spring it may be a millimeter or more in thickness. When hot weather returns the disease again becomes active, involving the new wood, and the plant eventually dies.

Further knowledge of the effect of temperature on the development of dwarf must await a better understanding of the cause of the disease. So far as can be determined from a study of the records of the areas where dwarf is known to exist, temperature is probably not a limiting factor in the spread of the disease. Neither can temperature be considered a causal factor, since in localities where dwarf does not occur temperatures are found that are higher or lower than temperatures in localities where dwarf is known to be most destructive.

EFFECT OF SOIL FERTILITY

Many investigations have been conducted to determine what effect soil fertility has on the susceptibility of plants to disease. The review of the literature on one small group of diseases, by Stakman and Aamodt (*11*), shows the consensus of opinion to be that nitrogenous fertilizers increase the susceptibility of cereals to rusts whereas potassium and phosphorus salts tend to decrease it. These writers state that there is no very close agreement among investigators as to the exact way in which fertilizers effect a change in the resistance of a plant to rust. Stakman and Aamodt consider the effect to be largely indirect, through changes in the growth rate, density of stand, date of maturity,

and yield. Much has been written regarding the correlation between the rapidity of growth of fruit trees and their susceptibility to fire blight. Hoffer and Carr (*6*) have shown how the susceptibility of corn to root rot is influenced by the accumulations of iron and aluminum in the nodes, and how the availability of these compounds in the soil may be influenced by soil moisture and the presence of sufficient amounts of other salts such as calcium and phosphorus. It has also been shown in recent years (*1, 2, 4, 7, 9, 13*) that many soils are deficient in certain elements, such as copper, manganese, zinc, and boron, which are needed in very small amounts by plants but which may be quite toxic, producing conspicuous symptoms, if excessive amounts are present in the soil. The results of such investigations serve to emphasize the fact that soil fertility may affect disease in plants by increasing or decreasing the growth rate or by weakening the tissue, as in the case of iron and aluminum, and that a deficiency or an excess of certain elements may produce a condition in plants resembling that caused by micro-organisms.

In 1929 experiments were initiated to determine whether the addition of certain commonly used fertilizers would influence the severity of the dwarf disease or whether the affected plants would outgrow the disease when supplied with certain elements that might be lacking.

The first experiment was designed to show whether the affected plants would outgrow the disease when they were removed from the field and planted in cans containing soil to which manure or commercial fertilizers had been added. For this experiment subsoil was taken from a mountain side where no cultivated crop had ever grown. Six 16-gallon garbage cans were used; two of them, containing only the soil, served as controls; two others were filled with soil mixed with a nearly equal amount of fresh horse manure; and the remaining two received an application of a complete fertilizer (consisting of ammonium sulphate, nitrate of soda, blood, triple superphosphate, and sulphate of potash), which supplied 7.5 percent nitrogen, 9.25 percent P_2O_5, and 4.75 percent K_2O, at the rate of 250 pounds per acre. The soil that was used supported vigorous growth without the application of any fertilizer. Eight 2-year-old alfalfa plants having an early stage of the disease were planted in each can. The experiment was set up in October 1929.

The plants used would normally have died during the summer of 1930. Observations made on October 22, 1930, showed that three of the control plants were alive but that their top growth was only about one third as tall as that of healthy plants growing in nearby cans and cut at the same time. A like number of plants in the cans to which manure had been added were alive and these too were very much dwarfed. Although the top growth was little if any better, the plants in the soil to which commercial fertilizer had been added did not die quite so rapidly, a total of seven plants being alive after 1 year. After 2 years one plant still remained alive in one of the cans treated with manure and one was alive in the soil to which commercial fertilizer had been applied. Just how long some of the control plants might have lived was not determined, as these plants were removed on December 2, 1930, and the cans used for another experiment.

It was concluded from this experiment that none of the treatments appreciably affected the rate of dying or the growth of the plants.

It is of course true that the experiment was carried out on a very limited scale; therefore one would probably not be justified in drawing any conclusions from it if the results had not been confirmed by field observations and other experimental data.

Although no fertilizer tests were conducted in the field, the writer had the privilege of observing and taking notes on such a test conducted during the years 1929–31, inclusive, by M. M. Winslow, farm adviser, of Riverside County, Riverside, Calif. In this experiment there were eight plots, each 50 by 80 feet in area, representing in duplicate three different treatments and a control. The rate per acre and the fertilizers applied were (1) 750 pounds of superphosphate, (2) 200 pounds of sulphur and 600 pounds of lime, and (3) 750 pounds of superphosphate and 500 pounds of potassium chloride. Since no difference in the vigor of the plants was observed, no yield data were taken.

TABLE 6.—*Number of healthy and diseased alfalfa plants in plots receiving different fertilizer treatments*

Fertilizer treatment		Alfalfa plants in—						Average percentage of diseased plants
		Plot 1			Plot 2			
Plant food	Quantity per acre	Healthy	Diseased	Total	Healthy	Diseased	Total	
	Pounds	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
Superphosphate	750	226	64	290	199	65	264	23.4
Sulphur	200	145	66	211	141	54	195	29.5
Lime	600							
Superphosphate	750	186	64	250	216	54	270	22.8
Potassium chloride	500							
Control		214	46	260	204	44	248	17.7

The conclusion reached by Mr. Winslow and the writer, namely, that none of the fertilizers had any appreciable effect on dwarf, is supported by the data presented in table 6, which were obtained in February 1931 by digging all the plants in two 6-inch strips extending across all the plots and located about one third of the distance from either end. Further counts were to have been made in the autumn, but the stand was so thin by that time that it was not deemed worth while. Table 6 gives the number of healthy and diseased plants and the average percentage of diseased plants in the duplicate plots. These values show a very small range in the percentages of diseased plants in the plots. There was practically no difference between the plots receiving superphosphate and those receiving both superphosphate and potassium chloride. The value for the lime-and-sulphur plots (29.5) probably is not significantly greater than the values for the other plots. On the other hand, the percentage of disease in both control plots was the lowest of all. In none of the plots was the ground quite level, so that at times there was a difference in soil moisture; this may account for some of the variation. Taking everything into consideration, it seems probable that none of the fertilizers had an appreciable effect on the disease; certainly they did not prolong the life of the stand. By the end of the third season the stand was so uniformly poor that it was no longer worth retaining.

As stated previously, it is known that certain chemicals, ordinarily used by plants in small amounts, may have a profound effect upon them. In order to determine whether such chemicals would affect the susceptibility of alfalfa plants to dwarf, three experiments were conducted in which the following chemicals were tested: Boron, manganese, strontium, bromine, titanium, iodine, aluminum, copper, and zinc. The use of these chemicals was suggested largely by an article by Haas and Reed (*5*) and also by Dr. Haas in a personal consultation. Chemically pure chemicals were used. For the most part these were sulphates or nitrates; boron, however, was applied as sodium borate, and iodine as potassium iodide. Magnesium sulphate and ammonium nitrate were also added to this mixture. Fifty grams of boron, 1 g of titanium, 1 g of iodine, and 454 g of other substances were applied to each of two plots, each 15 by 40 feet in area, in February 1930. In one plot, which was beginning its second year, about 10 percent of the plants were diseased; in the other, which was beginning its fourth year, about 40 percent of the plants were diseased. As a further control, a heavy application of cow manure was made on a portion of the field adjacent to the plot having the older stand. In each case there was plenty of alfalfa growing on adjacent untreated land to serve as a control. In order that the counts of the plants might be made from the same area each time, three permanent quadrats, each 3 feet square, were staked off in the plot having the younger stand. Very early in the season it became apparent that an unfortunate choice of location had been made for the plot having the older stand; hence only general observations were made upon this plot throughout the season. Careful observations throughout the summer showed no apparent difference between the areas to which the chemicals and the manure had been added and the control areas.

In the plot having the younger stand counts of the quadrats showed that an average of one third of the plants had died during the year. The loss of plants was irregular; 50 percent disappeared at one end of the plot and only 7.5 percent at the other. In February 1931 counts made of representative areas in the plot showed that an average of 40 percent of the remaining plants had dwarf. The plants died so rapidly during the summer of 1931 that comparatively few were left by autumn; hence no further counts were made. It was clearly evident that the chemicals had not increased the longevity of the stand. At no time was there sufficient difference between the plot and the surrounding stand to enable one to locate the former except by the stakes at each corner.

At the time that these two plots were laid out a small experiment was begun in which two 16-gallon garbage cans were filled with soil from one badly diseased field and two with soil from another badly diseased field. Two of the cans, one filled with soil from each field, were treated with a proportionate amount of the chemical mixture used on the plots; the other two cans were left untreated and served as controls. In each can 10 alfalfa plants, 5 healthy and 5 having an early stage of dwarf, were set in such a way that healthy and diseased plants alternated. The object of this procedure was to determine whether the healthy plants would become diseased and whether the diseased plants would recover. The plants were set out on February 19, 1930.

Two plants in each of two cans, one treated and one control, and one plant in each of the other two cans failed to recover from the shock of transplanting; the other plants grew nicely. By October 21, 1930, all the diseased plants, except two in one of the control cans, had died; and two of the originally healthy plants, one in a treated can and one in a control can, had typical dwarf. The experiment was discontinued on July 3, 1931, when all the plants either had died from dwarf or had some stage of the disease. In no case did plants affected with dwarf outgrow the disease or healthy plants remain healthy. Although none of these experiments were conducted on a very large scale, the results are in agreement and are quite clear-cut; they indicate that ordinary fertilizers and other chemicals used have little or no effect on the susceptibility of alfalfa plants to the dwarf disease, and prove fairly conclusively that dwarf is due neither to an excess nor to a deficiency of any of these substances.

SUMMARY

Data are presented in this paper which indicate that dwarf, a recently described alfalfa disease whose cause is yet unknown, is largely responsible for the rapid dying of alfalfa plants in southern California.

Plots irrigated twice each month during the growing season and cut regularly were worthless for commercial hay production by the middle of the fourth year, whereas other plots similarly treated but allowed to remain without irrigation during the seed-growing period had fair stands at the end of the fourth year. The experimental evidence obtained has confirmed field observations that soil moisture exerts a marked effect on the severity of dwarf and consequently on the premature dying of alfalfa in southern California. Where soil moisture is kept sufficiently low, dwarf is not a factor in alfalfa culture. It hardly seems probable, however, that it will be commercially practicable to keep the soil moisture low enough to control dwarf and yet to maintain production at a desirable level.

Cutting alfalfa plants in the bud stage hastened the thinning of the stands to some extent, but not sufficiently to be of much commercial importance.

The development of dwarf in the plant is influenced to some extent by seasonal temperature, but its distribution is probably not limited by temperature to the regions in which it now exists.

Soil type does not appear to be much of a factor in the severity of dwarf. Neither manure, superphosphate, lime and sulphur, potassium, boron, manganese, strontium, bromine, titanium, iodine, aluminum, copper, or zinc were effective in controlling dwarf or in prolonging the life of the stand.

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EFFECT OF STEAM STERILIZATION ON SUSCEPTIBILITY OF WOOD TO BLUE-STAINING AND WOOD-DESTROYING FUNGI[1]

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INTRODUCTION

It is the ordinary procedure in experimental study of fungus action on wood to sterilize with steam the material to be inoculated, the treatments varying from a single autoclaving of 20 minutes at atmospheric pressure to repeated pressure treatments of 30 minutes each. A number of workers have suspected that such heating, especially the more severe pressure treatments, may change the chemical composition of the wood to a degree sufficient to make the wood an abnormal substratum for staining and wood-destroying fungi. This paper compares through the medium of strength tests the effects of different steam treatments on the susceptibility of certain species of southern pine to blue-staining and wood-destroying fungi.

BLUE-STAIN STUDY

METHODS AND MATERIALS

In any comparative study of the strength properties of wood, the selection and matching of test specimens are important. It

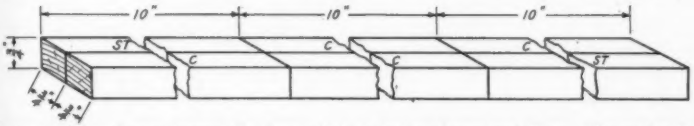

FIGURE 1.—A board sawn tangentially, from which six test specimens were taken. The specimens to be inoculated with stain fungi are marked ST; those kept as uninoculated controls are marked C.

was realized at the beginning of the study that since wood is a highly variable material, some knowledge of the comparative advantage of tangential and longitudinal matching should be had. In the first test, therefore, two control specimens, one tangentially and the other longitudinally adjacent, were taken for each specimen to be inoculated, as diagramed in figure 1. In this way the variability in strength properties of the control specimens alone could be determined and the different types of matching compared.

A statistical study of the variability of the strength values with the different types of matching was made, from which the advantage of matching specimens tangentially adjacent appeared to be only slightly greater than for specimens longitudinally adjacent. Both types of matching were therefore used in subsequent tests.

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[2] The writer wishes to express his appreciation to C. A. Richards and Carl Hartley, of the Division of Forest Pathology, and to T. R. C. Wilson, of the Forest Products Laboratory, for their valuable aid and criticism in the preparation of this paper.

Three species of wood, shortleaf pine, *Pinus echinata* Mill., loblolly pine, *P. taeda* L., and longleaf pine, *P. palustris* Mill., were used. All of the test specimens were cut from material taken at a height of from 8 to 16 feet in the tree, and carefully selected to get straight-grained wood of medium density and uniform growth. The bolts were split into sectors and the location of the specimens marked thereon in order to get true radial and tangential faces. All specimens were ¾ by ¾ by 10 inches in size.

The specimens were put into glass tubes at the bottom of which was placed distilled water to maintain a fairly high humidity and just enough broken glass to keep the stick from contact with the water. Two sterilization treatments were used, 30 minutes at atmospheric pressure and 5 minutes at 12 pounds' pressure. In two tests (nos. 4 and 5 of table 1) the specimens were sterilized outside of the tubes and transferred to sterile tubes inside a culture chamber. The same heat treatment was always given the matched controls except in the first two tests. In these tests the specimens to be inoculated were sterilized in glass tubes, whereas the control specimens were not tubed during sterilization. The latter group thus received the somewhat more severe heat treatment.

Some of the tests called for no steam treatment of the specimens. In these instances extreme care was exercised to keep contamination of specimens to a minimum. The planer and the saw were sterilized with mercuric-chloride solution and the specimens put immediately into sterile containers.

Inoculation consisted of spreading, inside a culture chamber, a heavy suspension of spores over the entire surface of each specimen. Two stain fungi were used, *Graphium rigidum* (Pers.) Sacc. (no. 198) and *Ceratostomella pilifera* (Fr.) Wint. (no. 185).

Complete coverage of each specimen with stain mycelium was usually obtained within 4 or 5 days. On the sticks that had not been sterilized this rapid development of mycelium kept mold contamination to practically nothing. Mold was observed to develop on only a few of the nonstained specimens; these were discarded.

The stain fungi were allowed to develop under optimum conditions of temperature and humidity for a period of 30 days before the specimens were tested. At the end of that time practically all of the specimens were stained throughout. Microscopic examination of the steamed and stained wood showed a greater abundance of fungus hyphae both in the ray cells and in the wood tracheids than is usually found in wood stained under lumber-yard conditions. Direct tracheid-wall penetration was also observed much more frequently than in normally stained wood, particularly with *Ceratostomella pilifera*. No marked change in color of the fungus hyphae from that found in unsteamed blue-stained wood was observed.

Three types of strength tests were made on most of the material—static bending, compression parallel to the grain, and toughness. The toughness test is a special test developed by the Forest Products Laboratory for testing small specimens. It is primarily a shock-resistance test, depending upon a combination of properties. All the specimens were tested in the green condition, in most cases well above the fiber saturation point.

RESULTS AND DISCUSSION

Table 1 presents the summarized results of the strength tests. Modulus of rupture and total work in bending were the values of static bending chosen for this summary, since they are of the most importance practically. Values for compression parallel to the grain are omitted. Values for toughness are given, since toughness is the strength quality most promptly affected by the action of fungi.

TABLE 1.—*Effect of steam disinfection on capacity of stain fungi to weaken wood*

Test no.	Stain fungus	Species of wood	Heat treatment	Number of specimens	Average values for inoculated wood expressed as percentages of average values for noninoculated wood subjected to the same heat treatment			
					Specific gravity (oven-dry weight)	Static bending		Toughness
						Modulus of rupture	Total work in bending	
1	*Graphium rigidum.*	*Pinus echinata.*	None	16	103	99	89	75
	Do	do	30 minutes at 100° C.	16	99	90	82	76
	Do	do	5 minutes at 12 pounds' pressure.	16	101	97	89	84
2	Do	*P. taeda*	None	25	98	96	92	77
	Do	do	30 minutes at 100° C.	25	104	96	67	63
3	Do	*P. palustris*	None	12	101	104	84	82
	Do	do	30 minutes at 100° C.	12	101	86	30	33
4	*Ceratostomella pilifera.*	do	None	12	100	100	49	57
	Do	do	30 minutes at 100° C.	12	100	100	37	38
5	Do	do	None	18	99	97	67	60
	Do	do	30 minutes at 100° C.	18	98	101	52	44
Summation [a]			None	83	99.5	98.4	77.7	70.2
			30 minutes at 100° C.	83	100.5	94.8	54.2	50.5

[a] The summation values used are the totals of the absolute strength values for the inoculated specimens expressed as percentages of the totals for the uninoculated.

Table 1 affords a comparison between steamed and unsteamed wood with respect to the effects of stain fungi on strength values. Here the average strength values for the inoculated unsteamed wood are expressed as percentage ratios of the values for the natural uninoculated unsteamed wood, and the average values for the inoculated steamed wood expressed as percentage ratios of the average values for uninoculated steamed wood. In other words, the factor of the direct effect of heat on mechanical properties, which, incidentally, appears to have been abnormally high in these experiments, is eliminated, and the table thus furnishes a direct comparison of the effect of stain fungi on wood with and without steam treatment.

Specific gravity was not perceptibly affected by either fungus. *Ceratostomella pilifera* had no apparent effect on modulus of rupture, but the *Graphium rigidum* sticks were lower in this value than the uninoculated in the heated wood in all three of the experiments; the decreases were so small that both their mathematical significance and their practical importance are doubtful. A very different result was obtained with the toughness test, and also with the static bending total work values, which are reflections of toughness more than of

strength. In these values the fungi caused an average reduction of approximately one fourth in unheated wood and nearly one half in wood that had been steamed. The reduction was somewhat greater in toughness than in total work, and the *Ceratostomella* seemed to have more effect on these values than the *Graphium*. The apparent difference between the fungi may have been increased by the fact that in test 1 some of the *Graphium* sticks were not fully infected; in any case a larger number of tests and tests with other strains of the fungi would be needed to establish the greater effectiveness of the *Ceratostomella*.

The results indicate that stain fungi may affect very appreciably certain of the mechanical properties of wood, and that the steam-sterilization treatments commonly used in experiments with fungi on wood decidedly increase the susceptibility of the wood to deterioration by stain fungi. It would not be safe to conclude from them that wood given a commercial heat treatment, such as kiln drying or steaming, is much more subject to deterioration by fungi than is natural unheated wood, or that the occurrence of stain in lumber would necessarily mean a pronounced loss in toughness of the wood. In this experimental work the conditions for stain development were optimum, and these conditions maintained for a period of 30 days were probably more conducive to deterioration by stain fungi than those ordinarily present in the lumberyard, where pronounced variations in humidity and temperature are usually encountered.

The chief value, then, of the results of these few tests lies not in respect to the effect of heat treatment of wood as lumber, but in the fact that they are significant to those studying the effect of fungi on wood. Conclusions that may be drawn from experiments in which the wood has been steam sterilized may be subject to considerable error unless due consideration of its effect is given in the interpretation of results. It would seem necessary to qualify the acceptance of some of the past experimental work that has been done with wood disinfected by heat.

Table 2 gives an idea of the variation, or change in the observed average, for the different treatments for the three tree species. A comparison of the coefficients of variation for unheated wood indicates that staining, as might be expected, is a highly variable factor and increased the variability of the wood in toughness strength; but in heated wood there was no indication of increased variability due to inoculation in either *Pinus echinata* or *P. taeda*. If any conclusion be drawn on so small a basis of evidence, it is suggested that some factor in the unheated sticks of all three pine species causes them to differ in their susceptibility to attack by the fungi used; but that in two of these species heat treatment by destroying inhibiting factors or increasing the digestibility to fungi of the cell walls has made the different sticks more nearly equal in susceptibility to deterioration than before heating.

TABLE 2.—*Effect of treatment on standard deviation and coefficient of variation in toughness test*

Treatment	Species of wood	Number of specimens	Mean	Standard deviation	Coefficient of variation
Natural, not steamed, not inoculated	*Pinus echinata*	16	280	57.2	20.4
	Pinus taeda	25	508	65.1	12.9
	Pinus palustris	24	515	77.6	15.1
Not steamed, inoculated	*Pinus echinata*	16	204	87.3	42.8
	Pinus taeda	25	400	96.2	24.1
	Pinus palustris	24	390	87.2	22.3
Steamed 30 minutes at 100° C., inoculated	*Pinus echinata*	16	184	34.4	18.8
	Pinus taeda	25	321	35.6	11.1
	Pinus palustris	24	205	77.5	38.0

DECAY STUDY

METHODS AND MATERIALS

Decay fungi were employed only in a single experiment. The material was green loblolly pine sapwood (*Pinus taeda*), cut into longitudinally alined specimens ⅝ by ¾ by 4 inches in size, with 2-inch samples for moisture determination distributed alternately between them. The moisture content of each 4-inch test specimen was assumed to be the same as the average of its two adjacent 2-inch samples, and its oven-dry weight was thus computed from its green weight. Alternate 4-inch test specimens were given one of two steam treatments—30 minutes autoclaving at atmospheric pressure, or 5 minutes at 12 pounds—and were compared with the intermediate control specimens that had been unheated. The unheated specimens were sawed out under as aseptic conditions as possible, and placed immediately on fungus mats in Kolle flasks; their green weight was taken as the average of the two adjacent specimens of the same size. Half of the specimens were placed on mycelial mats of *Poria incrassata* (Berk. and Curt.) Burt., and half on mats of *Lentinus lepideus* Fr., both of which decay pine at about the same rate. The percentage loss in weight was determined at the end of 30 days for part of the specimens, and at the end of 70 days for the remainder.

RESULTS AND DISCUSSION

As was expected, at the time of final inspection a number of the specimens (8 of the 18 unheated specimens) were contaminated with molds, which in all probability tend to inhibit growth of the wood destroyers. All the visibly molded specimens except one which was very lightly molded were discarded. The discarding of the molded specimens may have involved loss of the most susceptible wood. The presence of contamination also throws doubt on the accuracy of the results and prevents clear-cut conclusions being drawn. The results are presented, however, in the belief that they are indicative. Further work is necessary.

Table 3 indicates that after the first 30 days decay was relatively slower for the natural unheated specimens and that the 5-minute pressure treatment predisposed the wood to decay to a greater degree than the 30-minute treatment at atmospheric pressure. Roughly, there is a ratio of 3:2:1 for the amount of decay at the end of 70 days for the three types of treatment, 5 minutes at 12 pounds' pressure,

30 minutes at atmospheric pressure, and no steaming; all of which indicates that steam sterilization, especially with pressure and resultant higher temperatures, makes the wood a more suitable substratum for these wood-destroying fungi after the fungus has passed the earliest invasion stage.

TABLE 3.—*Percentage loss in weight of wood specimens due to decay*

Sterilization treatment	Period of decay, 30 days		Period of decay, 70 days	
	Specimens	Loss in weight	Specimens	Loss in weight
	Number	*Percent*	*Number*	*Percent*
5 minutes at 12 pounds' pressure	15	2.14	21	19.95
30 minutes at atmospheric pressure	13	3.13	11	11.49
None	11	2.16	20	6.77

WOOD STERILIZATION METHODS

It appears that other sterilization methods must be employed if the wood used in laboratory experiments is to simulate wood in the natural state. A number of methods have been suggested and tried, most of them chemical, involving the use of volatile toxic materials, but with little assurance that their application does not change the composition of certain constituents of the wood.

SUMMARY

From the results of the experiments with heated and unheated pine wood on the effect (1) of staining fungi on strength properties and (2) of wood-destroying fungi on the loss in weight of wood, it appears that even the relatively mild treatments ordinarily used in disinfecting blocks for wood-decay experiments may so change the wood as to make it a more congenial substratum than normal unheated wood for at least some of the wood-deteriorating fungi. Consequently, any conclusions that may be drawn from experiments in which the wood has been steam sterilized are subject to considerable error, unless due consideration is given to the effect of such sterilization in the interpretation of results. In view of the evidence submitted here, it seems necessary to qualify the acceptance of some of the past experimental work that has been done with wood disinfected by heat.

SOME PHYSIOLOGICAL EFFECTS OF ULTRAVIOLET RADIATION ON HONEYBEES[1]

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INTRODUCTION

The beneficial effects of ultraviolet light on higher animals such as chickens, rats, and human beings are now well known. It has been used with great success in the treatment of such diseases of man as tuberculosis of the skin, bones, or viscera, skin diseases of various kinds, rickets, and tetany. Not so well known, perhaps, are the harmful effects of ultraviolet light on various organisms. Bacteria, for example, are readily killed by the so-called "middle ultraviolet", that is, by radiations of wave lengths between 210 and 297 mμ [3]; plants are rather generally harmed by wave lengths shorter than 290 mμ [4]; and animals, including man, may suffer from serious burns or internal disorders from it under certain conditions.

The possibility of finding in ultraviolet light a source of benefit to bees, and consequently to the beekeeping industry, has led to a few investigations on the physiological effect of these rays on honeybees. Probably the first was that performed by two Hungarian workers, Stitz and Beyer, in 1927.[5] These workers irradiated one colony of bees and kept another, as nearly identical as possible, as a control. They describe the method and results of their experiment in part as follows:

* * * Naturally the main experiment was carried on for several weeks, always with intensive irradiation. The combs with the bees were irradiated individually and were always checked against the other nonirradiated colony, which was equally strong and handled otherwise in exactly the same way. The result was startling. The days after the irradiation one recognized at once, "among 100 colonies", the irradiated ones by their heightened activity and their active coming and going. Moreover, metabolism was decidedly stimulated. The irradiated colonies built about 1½ times as many new cells as the controls, but most of all was to be noticed the action of ultraviolet rays on the development of the larvae. Their development was shortened by two days. When the irradiation was overdone—as took place in the last test of the main experiment—the bees came out of their cells before they were completely developed, so that their abdomens were still entirely white. (Naturally, these individuals died after a short time.) * * * One can therefore conclude that the influence of ultraviolet rays is favorable to the different life phenomena of bees.

The only other work done along this line, so far as the writer is aware, is that of Muth and Balinkin [6 7] in the United States. This

[1] Received for publication Feb. 4, 1933; issued October 1933.

[2] The writer wishes to express his gratitude to Jas. I. Hambleton, in charge of the Division of Bee Culture, Bureau of Entomology, U.S. Department of Agriculture, where the present investigation was made, for facilities granted and for many useful suggestions; and to Rockwood Nusbaum, commercial beekeeper of Union Bridge, Md., with whose cooperation some of the experiments on queens were carried out.

[3] ELLIS, C., and WELLS, A. A. THE CHEMICAL ACTION OF ULTRAVIOLET RAYS. 362 p., illus. New York. 1925.

[4] ARTHUR, J. M., and NEWELL, J. M. THE KILLING OF PLANT TISSUE AND THE INACTIVATION OF TOBACCO MOSAIC VIRUS BY ULTRAVIOLET RADIATION. Amer. Jour. Bot. 16: 338–353, illus. 1929.

[5] STITZ, J., and BEYER, M. DIE BIOLOGISCHE WIRKUNG DER ULTRAVIOLETTEN STRAHLEN—AUF DIE BIENEN. Arch. Bienenkunde 8: 286–288. 1927.

[6] MUTH, C. F. ULTRA VIOLET RAY AND QUEENS. Beekeepers Item 11(5): 147–148. 1927.

[7] ——— ULTRA VIOLET RAYED QUEENS. Iowa State Apiarist Rpt. 1928: 59–64, illus. 1928.

work was started in 1926 and apparently has been continued ever since. These men claim to have noted such decidedly beneficial effects on the queens as a result of the treatment that they have applied for a patent on their process and have placed irradiated queen bees on the market, selling them for a higher price than ordinary queens. According to the meager data which they published,[8] they used first a carbon arc and later a quartz mercury-vapor lamp, filtered the rays through a glass filter which "shut out the wave lengths less than 2,700 angstrom-units", and, in the only one of their successful experiments for which the length of exposure is stated, gave the queens a 15-second exposure on the first day, no exposure on the second day, a 30-second exposure on the third day, no exposure on the fourth day, and a 90-second exposure on the fifth day. Neither the distance from the lamp nor the size of the lamp is stated.[9]

> With this experiment there was a steady increase in egg-laying on all of the treated colonies until at the sixth week there was a 30 percent increase in egg-laying over and above that of normal.

They also found that the progeny of the treated queens were much more gentle than ordinary colonies, and that even Cyprian bees, which are notorious for their bad temper, were made gentle by having come from a treated queen.

Muth and Balinkin [8] also treated some queen larvae with ultraviolet rays.

> We treated a young queen cell all during its period of development, keeping the frame which contained this queen cell in our office. * * * When this queen emerged * * * she was without doubt the largest queen that my father, several beekeepers who have seen her, and myself have ever witnessed in our lives.

These reports are very interesting, and, if they are confirmed by subsequent work, treatment of bees with ultraviolet rays ought to have both a theoretical and a practical importance—theoretical in that it may lead to a better understanding of the physiological action of ultraviolet light, and practical in that it may enable us to increase the honey crop and perhaps eventually to apply the results to other insects or other animals.

To extend the investigations already made and to check the results more carefully, the experiments described in this paper were performed. The effect of ultraviolet rays was tested first on adult workers and then on worker larvae, since such material is plentiful, and finally the knowledge thus gained was applied to the more limited supply of queens, both adults and larvae.

EFFECT OF ULTRAVIOLET RADIATION ON THE LONGEVITY OF ADULT WORKERS

Of the various criteria for ascertaining the physiological effect of ultraviolet radiation on an animal, longevity is one of the easiest to measure and is probably as good an indication of the total physiological effects as any. It was therefore chosen in this series of experiments.

In the first experiment 1-day-old adult workers were used, but they lived for such a short time that in subsequent experiments slightly

[8] MUTH, C. F. See footnote 7.

[9] Since this report was written the patent applied for has been issued (U.S. Patent 1,868,042; issued July 19, 1932). In the application for patent (Mar. 23, 1929) some additional data are given as to the method of treatment. The queens are placed "around 39 inches" from the lamp and exposures of "around one minute, two minutes and five minutes", respectively, are made on 3 successive days.

older bees were used. These were obtained by shaking the bees from a frame onto the ground and picking up those that did not fly away. This assured one of getting fairly young bees (ordinarily less than 2 weeks old), since the older ones, the field bees, flew away at once. As the bees were picked up they were put into a specially designed cage, 50 bees to a cage.

The bottom and the top of the cage (fig. 1) were made of heavy copper-wire screen, and the sides and ends of wood. Over the vestibule at the front of the cage was a sliding cover provided with a hole into which was inserted the open end of a large test tube containing queen-cage candy. The cover could be taken out for cleaning by sliding it forward, or it could be slid back far enough to permit removal of the dead bees with forceps after they had been shaken down to the

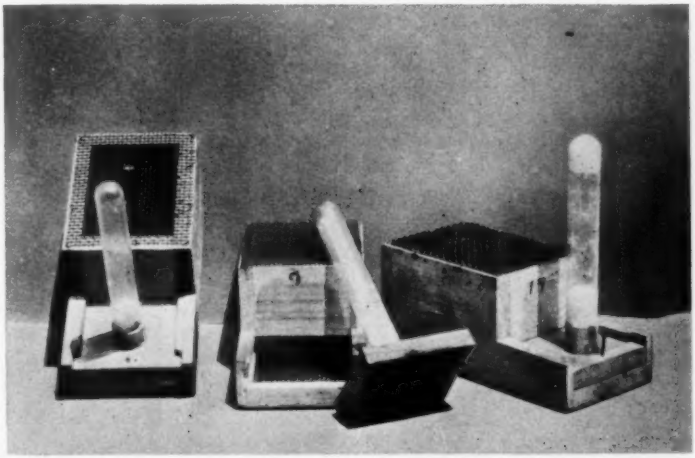

FIGURE 1.—Three views of the cage used for keeping adult worker bees in confinement and for exposing them to light. The glass tube contains queen-cage candy for food.

front. The cages, when new, were soaked in hot paraffin; hence it was easy to clean them with soap and water before starting an experiment. During an experiment they were kept lying on one side, thus holding the candy tube in a horizontal position so that the bees, after becoming feeble, could get food easily.

The irradiation was done through the bottom of the cages. A quartz mercury-vapor lamp (Uviarc), 220-volt size (fig. 2), was used and was run at about 4 amperes. It was provided with a hood, which also served as a reflector. At the front of the hood was a cover having an opening 6 inches square, over which any desired filter could be placed. During the course of the investigation three glass filters—Pyrex, Corex D, and Corex A red-purple—were used. The quality and quantity of light transmitted by these filters, given in table 1, were measured by R. Stair, of the United States Bureau of Standards. The intensity of irradiation 31.5 inches from the lamp was sufficient to produce a decided erythema on untanned skin in about 1 minute.

TABLE 1.—*The quality and quantity of radiant energy emitted by the quartz mercury-vapor lamp and transmitted by three glass filters*

Filter used	Lower limit of spectrum emitted or transmitted	Upper limit of spectrum emitted or transmitted	Relative energy emitted or transmitted
	Millimicrons	*Millimicrons*	*Percent*
Open lamp, no filter	185	1,400+	100.0
Corex D	297	1,400+	24.5
Pyrex	313	1,400+	22.6
Corex A red-purple	265	405	4.7

The cages of bees were examined daily except Sundays, and all dead bees were counted and removed. Dead bees were assumed to

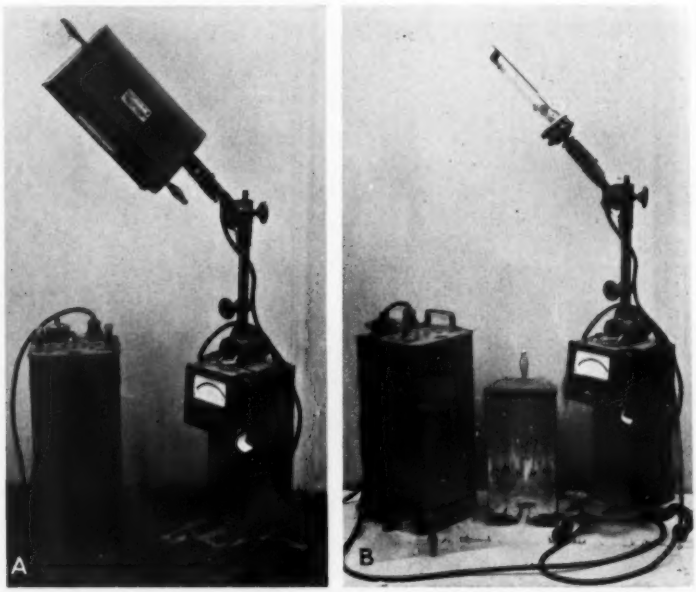

FIGURE 2.—Two views of the quartz mercury-vapor lamp and accessories used in these experiments: A, With lamp house and filter in place; B, with lamp house and filter removed. The large apparatus at the left is a rectifier for making direct current from alternating current.

have died midway between the time at which they were found dead and the preceding examination of the cage, and the mean longevity for the entire 50 bees in each cage was computed on this assumption. The probable error of the mean was computed according to Peters' formula

$$r_0 = \frac{0.8453\Sigma v}{n\sqrt{n-1}}$$

where v = the variation of the length of life of each bee in the cage from the mean for the whole cage, and n = the number of bees in the cage.

Except while the bees were being counted or irradiated, the cages were kept in a dark room maintained at about 21° C. (70° F.). Six experiments were performed in the manner outlined above.

EFFECT OF LIGHT FROM OPEN LAMP

The effect of the light from the open lamp in exposures of various length was first tested. The bees were all very young, 1 day old or less. The cages were placed 12 to 14 inches from the lamp and given exposures varying from a single exposure of 30 minutes down to a 10-second exposure on 3 different days. The results are presented in table 2. It will be noted that in no case did the bees in the treated lots live as long as the untreated controls. The fact that these bees were very young, however, and that the distance from the lamp was so short that there was a considerable radiation of heat (the exact temperature was not taken) may have had more influence on longevity than had the light.

TABLE 2.—*The longevity of adult honeybees, 1 day old or less at the start of the experiment, when exposed at a distance of 12 to 14 inches for various lengths of time to the unfiltered light from a quartz mercury-vapor lamp, as compared with the longevity of untreated bees*

Cage no.	Treatment	Maximum longevity	Average longevity
		Days	*Days*
5	Control, untreated	14	6.74±0.20
6	30 minutes first day only [a]	10	3.99± .20
10	15 minutes first and second days only	6	2.24± .05
11	5 minutes daily [b]	6	3.38± .13
12	1 minute daily [b]	11	5.87± .16
15	15 seconds daily [b]	13	5.65± .23
17	5 seconds daily [b]	7	3.29± .11
22	30 seconds first, second, and fourth days each	12	6.22± .20
23	10 seconds first, second, and fourth days each	9	3.22± .17
25	30 seconds first day, 1 minute second day, 1½ minutes fourth day	10	4.32 ±.18

[a] Temperature at cage at end of this period was 43° C. (109° F.).
[b] As long as any bees in the cage remained alive.

TABLE 3.—*The longevity of adult bees, about 1 week old at the start of the experiment, when exposed at a distance of 31.5 inches for various lengths of time to the unfiltered light from a quartz mercury-vapor lamp, as compared with the longevity of untreated bees*

Cage no.	Treatment [a]	Maximum longevity	Average longevity
		Days	*Days*
4	Control, untreated	21	14.72±0.26
8	30 minutes	14	7.59± .32
10	15 minutes	14	7.70± .32
11	5 minutes	23	7.30± .37
13	1 minute	25	17.62± .41
18	½ minute	28	18.45± .36

[a] All treatments were on alternate days as long as any bees remained alive in the cage.

A second experiment was therefore performed in essentially the same way except that slightly older bees, about 1 week old, were used, the distance from the lamp was increased to 31.5 inches, and exposures were made on alternate days as long as any of the bees in the cages remained alive. This time the highest temperature during exposure was 35° C. (95° F.) as compared with 30° C. (86° F.) prevailing in the room. This difference was probably not enough to affect longevity appreciably. The results are presented in table 3.

In this experiment exposures of 30, 15, and 5 minutes on alternate days were all decidedly harmful to the bees, but exposures of 1 minute and one half minute on alternate days were beneficial.

EFFECT OF THE ULTRAVIOLET PORTION OF THE LIGHT

The result of this experiment suggested the question as to whether the benefit apparently caused by the light was due to its short-wave, ultraviolet portion or to the longer wave lengths. To answer this question it was decided to use a filter for the light which would cut off the longer radiations and transmit only the ultraviolet. This was easily done by using the Corex A red-purple filter, which transmits only wave lengths between 265 and 405 mμ (table 1). The exposures were made as in the preceding experiment, that is, on alternate days and at a distance of 31.5 inches from the lamp. The lengths of exposure were 1, one half, and one fourth minute each, since the shorter periods had seemed to be beneficial in the previous experiment.

The results are presented in table 4. None of the treated bees lived longer than the controls, and the only ones that lived as long as the controls were in the cage exposed one fourth minute. This indicates that, if any of the light from the quartz mercury-vapor lamp is beneficial, it must be either the light of a wave length longer than 405 mμ or of one shorter than 265 mμ.

TABLE 4.—*The longevity of adult bees when exposed at a distance of 31.5 inches for various lengths of time to the light from a quartz mercury-vapor lamp filtered through a Corex A red-purple filter, as compared with the longevity of untreated bees*

Cage no.	Treatment [a]	Maximum longevity	Average longevity
		Days	*Days*
6	Control, untreated	16	9.79±0.46
12	1 minute	19	7.22± .66
17	½ minute	19	5.89± .52
23	¼ minute	16	9.74± .54

[a] All treatments were on alternate days as long as any bees remained alive in the cage.

EFFECT OF LIGHT OF VARIOUS WAVE LENGTHS

In order to determine whether it is the very short wave lengths or those longer than 405 mμ that cause the apparent increase in longevity, exposures were made to light filtered through Corex D, Pyrex, and Corex A red-purple filters, respectively, in all treated cages except the one irradiated with the open lamp for comparison. As indicated in table 1, Corex D and Pyrex cut off wave lengths shorter than 297 and 313 mμ, respectively, but transmit the longer waves freely. The length of each exposure was either 1 minute or one half minute, and exposures were made on alternate days and at a distance of 31.5 inches as before. The results are presented in table 5. All the irradiated bees lived as long as the controls, but none significantly longer except those irradiated by unfiltered light, which outlived the controls by an average of about 4 days. In this lot also the maximum longevity was remarkably great, one bee living 35 days—14 days longer than the best among the controls.

TABLE 5.—*The longevity of adult bees when exposed at a distance of 31.5 inches for various lengths of time to the light from a quartz mercury-vapor lamp, both unfiltered and filtered through three specified filters, as compared with the longevity of untreated bees*

Cage no.	Treatment *	Maximum longevity	Average longevity
		Days	*Days*
4	Control, untreated	21	14.16±0.30
5	Open lamp, ½ minute	35	18.65± .71
6	Corex D filter, 1 minute	24	14.26± .56
8	Corex D filter, ½ minute	23	14.25± .29
10	Pyrex filter, 1 minute	25	14.80± .55
15	Pyrex filter, ½ minute	27	16.06± .38
22	Corex A filter, 1 minute	23	15.38± .41
23	Corex A filter, ½ minute	24	14.74± .46

* All treatments were on alternate days as long as any bees remained alive in the cage.

Since the increased longevity under the unfiltered light is not due to radiations between 265 and 405 mμ, as shown by the results in cages 6 and 8 (also indicated in the previous experiment, table 4), and since it is evidently not due to wave lengths longer than 297 mμ (cages 22 and 23) or 313 mμ (cages 10 and 15), the conclusion is reached that, if it is due to any part of the light, it must be due to wave lengths shorter than 265 mμ. This conclusion is unexpected because neither in the experiments on other animals nor in those on plants has it been found, so far as the writer is aware, that radiations of wave length shorter than about 290 mμ are beneficial. These rays are not present in sunlight and are commonly called the "abiotic" or lethal rays.[10]

EFFECT OF EXPOSURE TO LIGHT OF VARIOUS WAVE LENGTHS BUT OF CONSTANT QUANTITY

There is another possible explanation of these results, however. Perhaps the increased longevity under radiation from the open lamp (table 5) is due, not to the quality of its light, but only to the quantity. With all cages placed 31.5 inches from the lamp, the one irradiated without a filter received much more energy than the others, and this may have been the optimum for greatest benefit to the bees. In order to test this explanation an experiment was performed in which the cages were placed at such distances from the lamp that the quantity of energy coming to each was the same, as ascertained by the transmission data given in table 1.

A rack made for this purpose was placed before the lamp, as shown in figure 3. Each cage was held on the inclined board by nails driven at such distances that when irradiated with light from the open lamp the center of the cage was 72.6 inches from the lamp tube, and when irradiated with light filtered through the Pyrex, Corex D, and Corex A glasses, at 34.5, 35.9, and 15.7 inches, respectively. All the cages except the controls were given 1½ hours' total exposure, divided as indicated in table 6. As the tests were made on duplicate cages, there were 100 bees in each lot instead of 50 as previously.

The results, presented in table 6, show that no lot lived significantly longer than any other. It is therefore fairly certain that the increased

[10] ELLIS, C., and WELLS, A. A. See footnote 3.

longevity observed previously in some of the cages was not due to the quality of the light received in those cages. It may be possible that the bees in those cages received a certain optimum quantity of energy, or that the increased longevity observed was not a real increase but

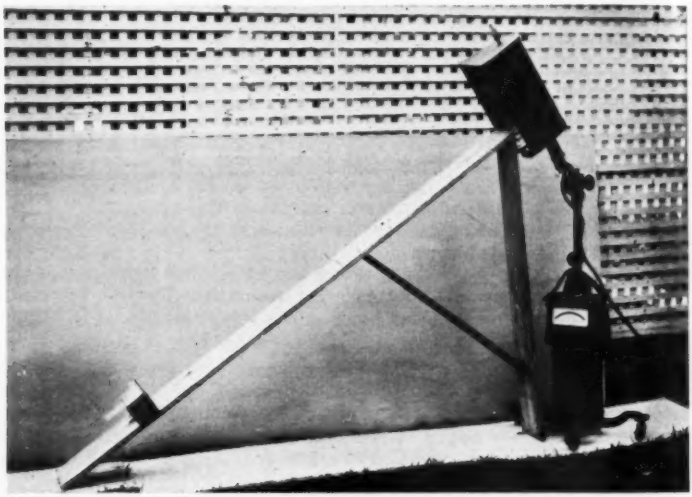

FIGURE 3.—Rack used for holding the cages of adult bees while exposing them to the light, shown in place before the lamp. The cage rests on nails driven part way into the board.

was wholly within the limits of experimental error. The writer is inclined toward the latter conclusion.

TABLE 6.—*Longevity of adult bees when exposed on the first nine consecutive days for 1, 2, 3, 4, 5, 10, 15, 20, and 30 minutes, respectively, to the light from a quartz mercury-vapor lamp, both unfiltered and filtered through three different filters at such distances that the energy coming to each cage was the same, as compared with the longevity of untreated bees*

Cage nos.	Treatment	Maximum longevity	Average longevity
		Days	*Days*
31 and 32	Controls, untreated	18	10.66±0.15
9 and 13	Open lamp at 72.6 inches	18	10.96± .22
14 and 15	Pyrex filter at 34.5 inches	17	10.98± .26
18 and 21	Corex D filter at 35.9 inches	18	11.23± .31
25 and 30	Corex A filter at 15.7 inches	17	11.05± .24

EFFECT OF IRRADIATION OF THE BEES' FOOD

There remains, however, still another possible explanation. Perhaps the light does not affect the bees directly, but only their food, and irregularities in the shading of the tube of food by the bees' bodies during irradiation may account for the discrepancies in the results of the different experiments. To test this explanation the food (queen-cage candy) for one cage of bees was spread out in a thin

layer and irradiated for 1 hour at 31.5 inches from the open lamp before being placed in the tube. Bees fed on this food were compared with others fed on nonirradiated food. The results, given in table 7, show that the controls lived slightly longer than the bees fed on irradiated food. Evidently, then, the irradiation of the food has no significant influence on the longevity of bees.

TABLE 7.—*The longevity of bees fed on candy that had been irradiated by the unfiltered light from a quartz mercury-vapor lamp, as compared with the longevity of bees fed on nonirradiated food*

Cage no.	Treatment	Maximum longevity	Average longevity
		Days	*Days*
6	Control, food untreated	16	9. 79±0. 46
25	Food irradiated	19	7. 68± . 52

SUMMARY OF RESULTS ON ADULT WORKER BEES

The general result of these experiments is, therefore, that direct irradiation of adult worker bees, whether by the total light from a quartz mercury-vapor lamp, or by the portion of the light above 313 mμ wave length, or by the portion above 297 mμ, or by the portion between 265 and 405 mμ, within the fairly wide limits of dosage used, was ineffective in increasing the length of life of bees in confinement but decreased the length of life of bees exposed for longer intervals, and that irradiation of the food only was likewise ineffective in increasing longevity.

SOME EFFECTS OF ULTRAVIOLET LIGHT ON WORKER LARVAE

In view of the claim by Stitz and Beyer [11] that the developmental period is shortened by irradiating the brood with light from the quartz mercury-vapor lamp, and the probability that larvae, with their thin sclerotic covering, are much more susceptible than adults to influence by these rays, it became especially interesting to investigate the effect of ultraviolet light on the larvae.

Broods at nearly every age were used in one or another of the experiments, but particularly larvae 2 to 3 days old. They were irradiated in the combs by taking the frame containing them out of the hive, shaking off all adult bees, and holding the frame in front of the lamp. The lamp used in the first few experiments was a carbon arc with Therapeutic C carbons (a carbon impregnated with iron, nickel, and aluminum, the arc of which is especially rich in ultraviolet light), 8 mm in diameter, and was run at 5½ amperes and 35 volts alternating current. For the other experiments the quartz mercury-vapor lamp already described was employed. The filters used were those given in table 1.

Usually only a single exposure was given, but sometimes a second exposure was given 1 day after the first. Opaque screens of various kinds were placed over portions of the brood area to shield these larvae from the light so that such areas could be used as controls. At the time of irradiation the larvae in each area were counted.

[11] STITZ, J., and BEYER, M. See footnote 5.

Examinations were then made at intervals of from one to several days to note the effect, and finally in some experiments upon emergence to note whether the irradiated brood emerged sooner than the controls. In one experiment the emerging bees were weighed to see if they differed in size from the controls. The details of each experiment are best described in connection with the results.

DOSAGE OF LIGHT LETHAL TO LARVAE

The first experiment was exploratory in nature, designed to see how great a dosage of the light from the carbon arc was necessary to kill the larvae. Five holes, each 2 inches square, were cut in an opaque screen the size of an ordinary frame in a beehive. This screen was placed over a frame of brood, the larvae of which were 1½ to 2½ days old, and the areas exposed through the holes were irradiated for one fourth, one half, 1, 3, and 10 minutes, respectively, at 7.8 inches from the arc. The larvae in each irradiated area were counted at the beginning of the experiment and again after 1, 2, 3, 4, 5, 7, 9, 10, and 12 days.

The results are shown in table 8. It will be noted that at all exposures except one fourth minute virtually all the brood was killed within 12 days, and that even at this exposure 80 percent were killed; that is, 80 percent of the brood were removed from the cells by the bees, and it is therefore assumed that they were killed by the light. The total effect is shown strikingly in the photographs of the frame both with and without the screen (fig. 4). Although no count of the larvae present on succeeding days was made on the nonirradiated areas, the photograph of the entire frame (fig. 4, A) shows that at least 90 percent of the larvae there survived.

TABLE 8.—*The lethal effect on brood, 1½ to 2½ days after hatching, of the unfiltered light from an electric carbon arc using Therapeutic C carbons, operated at 5½ amperes, 35 volts alternating current, and placed 7.8 inches from the comb*

Area irradiated	Duration of irradiation	Larvae present at beginning	Percentage of original number alive after indicated number of days								
			1	[a] 2	3	4	5	7	9	10	[b] 12
	Minutes	*Number*									
a	¼	85	100	72	41	41	41	38	27	27	20
b	½	95	100	47	38	35	33	4	3	3	1
c	1	115	99	9	6	4	3	1	1	1	1
d	3	115	94	[c] 48	33	27	20	6	3	3	1
e	10	115	3	3	3	3	3	2	2	2	1

[a] Most cells containing larvae were capped over on this day.
[b] Photographs taken on this day are shown in figure 4.
[c] The higher percentages in this area than in *c* are probably due to the fact that these larvae were slightly older than those in *c*.

In another experiment the effect of a 5-second exposure to the same light at a distance of 7.8 inches, on broods of different ages, was tested. The eggs, larvae just hatched, and larvae 1 day old were all killed by this treatment, but larvae 2 days old were not.

The surprising thing shown by these two experiments was the remarkably small resistance of brood to the light from this carbon arc. It was thought that perhaps this light was too rich in ultraviolet or in infrared rays, or for some other reason could not be com-

pared with that from the mercury-vapor lamp used with such success by Stitz and Beyer. Hence, the quartz mercury-vapor lamp (Uviarc) was brought into use. After some preliminary tests the following experiment was made:

Larvae of definite ages were obtained by confining a queen bee overnight in a part of the hive in which there was only a single empty comb. During the night she laid eggs in a considerable area on both sides of this comb. In the morning she was moved to another part of

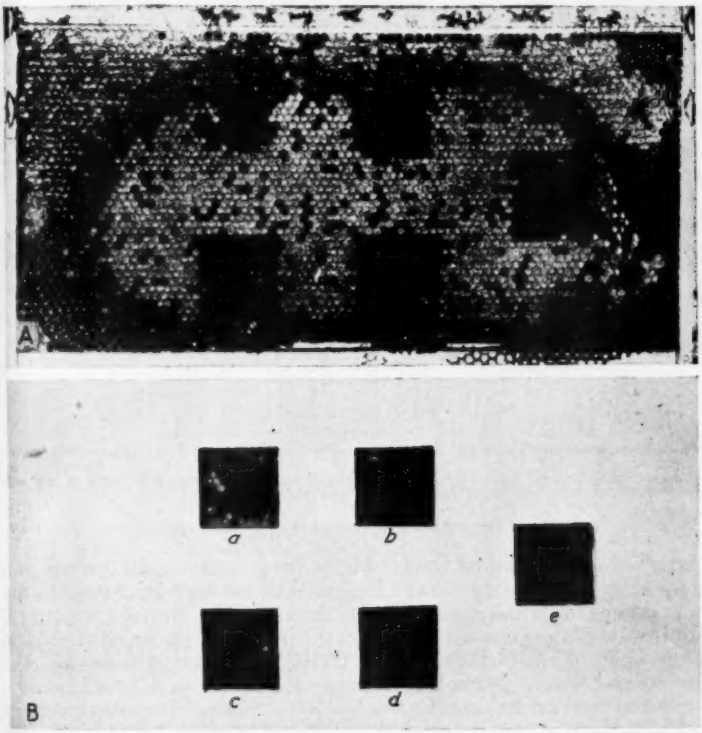

FIGURE 4.—Photographs of a frame of sealed brood in which various areas had been exposed to ultraviolet light while the larvae were 1½ to 2½ days old: A, The frame without the screen; B, the frame covered by the screen containing the holes through which exposures were made. The holes in the screen are lettered *a* to *e* as in table 8.

the hive, where she could not get to this comb again, and the eggs in the comb were allowed to develop. When the resulting larvae were 3½ days old (6½ days after the eggs were laid), the comb was taken from the hive, one side was covered with the screen already described (fig. 4, B), and four of the areas lying under the holes in the screen were given irradiations of 5, 10, 15, and 20 seconds, respectively, 31.5 inches from the mercury-vapor lamp. After the larvae in each irradiated area were counted, the comb was replaced in the hive. The next day the other side, with larvae now 4½ days old, was given the

same treatment. The irradiated areas were examined 1, 2, 5, 9, and 12 days after their irradiation.

The results presented in table 9 show that, although there was considerable variation in the death rate, yet after 12 days all the irradiated larvae, except 70 percent of those 4½ days old that were given the 5-second treatment, were dead. Apparently, then, the light from the mercury-vapor lamp is just as harmful to the brood as is that from the carbon arc. Observations as to the time of emergence of these 4½-day-old larvae which survived the 5-second treatment gave no evidence that they emerged as adult bees any sooner or later than did bees in nonirradiated areas of the same comb.

TABLE 9.—*The lethal effect on brood, 3½ and 4½ days after hatching, of the unfiltered light from a quartz mercury-vapor lamp run at 4 amperes and 140 volts direct current and placed 31.5 inches from the comb*

Age of larvae (days)	Area of comb irradiated [a]	Duration of exposure	Larvae treated	Percentage of original number alive after indicated number of days				
				1	2	5	9	12
		Seconds	*Number*					
3½	*a*	5	17	12	1	0	0	0
	b	10	28	61	54	21	0	0
	c	15	27	33	19	8	0	0
	d	20	25	0	0	0	0	0
4½ [b]	a^1	5	27	100	93	90	70	[b] 70
	b^1	10	10	100	90	80	10	0
	c^1	15	17	47	35	1	0	0
	d^1	20	32	94	88	66	0	0

[a] The areas designated as a^1, b^1, c^1, and d^1 were on the opposite side of the comb to the areas designated as *a*, *b*, *c*, and *d*.

[b] Examination of the comb while these bees were emerging showed them to be coming out of their cells neither sooner nor later on the average than their nonirradiated sisters.

WAVE LENGTH OF LIGHT INJURIOUS TO LARVAE

After it had been found that the light from both the carbon arc and the mercury-vapor lamp is very injurious to bee larvae, the next step was to determine what portion of this light contains the injurious rays. With the aid of the glass filters used in the investigation on adult bees it was possible to select that portion of the light having wave lengths longer than 313 mμ (Pyrex filter), longer than 297 mμ (Corex D filter), or between 265 and 405 mμ (Corex A filter). These filters were used in irradiating larvae just hatched and larvae 1, 2, and 3 days old. All exposures were of 5 seconds' duration, and in all cases the comb was placed 31.5 inches from the lamp.

The results, given in table 10, show clearly that the light from the open lamp was the most harmful, that the light filtered through the Corex A glass was next most harmful, and that the light filtered through Corex D and Pyrex glass had a much less harmful effect. The light filtered through the Corex D glass was most harmful for larvae 1 and 2 days old, and that filtered through the Pyrex was the most harmful for the larvae just hatched or 3 days old.

The results of this experiment show that the harm done is not entirely a matter of total energy reaching the larvae; for although they received the most energy from the open lamp, they received the least from the light filtered through the Corex A filter, and the

light filtered through the latter did more harm than that filtered through either Corex D or Pyrex. However, to verify this, an experiment was performed in which the actual energy received by all the larvae was the same. Larvae of known age were obtained as already described. The comb was divided into areas at the time of irradiation by affixing an opaque screen over all except the area to be exposed. By using the rack illustrated in figure 3, the area of brood to be irradiated was placed at 72.6, 34.5, 35.9, or 15.7 inches from the lamp, depending on whether no filter at all, Pyrex, Corex D, or Corex A was used. All the larvae were 2 days old at the beginning of the experiment and were given 15 seconds' exposure then and 45 seconds' exposure on the following day. Counts of the individuals alive at the time of the last exposure and after 1, 2, 4, 7, 9, and 13 days showed whether or not the light had a lethal effect. The results are given in table 11.

TABLE 10.—*The lethal effect on brood of different ages of 5 seconds' exposure to the total light from a mercury-vapor lamp, run at 4 amperes and 140 volts, and to three different spectral portions of it. Exposures made at 31.5 inches from the lamp*

Age of larvae	Filter used	Number of larvae treated	Percentage of original number alive after indicated number of days		
			2	5	13
Just hatched	None	21	100	15	9
	Pyrex	50	92	65	60
	Corex D	37	100	92	86
	Corex A	25	92	52	52
1 day	None	34	59	42	38
	Pyrex	34	100	94	94
	Corex D	20	90	75	75
	Corex A	18	94	39	39
2 days	None	24	0	0	0
	Pyrex	30	93	93	93
	Corex D	20	80	75	75
	Corex A	23	78	74	74
3 days	Pyrex	14	50	50	50
	Corex D	9	77	77	77

TABLE 11.—*The lethal effect on honeybee larvae, 2 days old at the beginning of the experiment, of a total exposure of 1 minute and 15 seconds on the first day and 45 seconds 24 hours later, to the unfiltered light from a quartz mercury-vapor lamp, run at 4 amperes and 140 volts, and to three different spectral portions of this lamp, at such distances that the total energy reaching the larvae was the same in all exposures*

Area irradiated	Filter used	Distance of comb from lamp	Larvae treated	Percentage of original number alive after indicated number of days					
				1	2	4	7	9	13 [a]
		Inches	*Number*						
a	None		56	38	16	14	4	0	0
b	Untreated control	31.5	144	100	100	99	77	77	77
c	Pyrex	34.5	91	99	98	98	98	98	97
d	Corex D	35.9	148	100	100	100	92	92	91
e	Corex A	15.7	179	35	2	2	1	1	1

[a] Photograph of frame taken on the thirteenth day shown in fig. 5.

Both the unfiltered light and the light filtered through the Corex A glass killed practically all the larvae, while the light coming through the other glasses killed practically none of them, for no more died here than in the untreated area. This is shown strikingly in photographs taken of each side of the frame on the thirteenth day (fig. 5). Note that the brood exposed to the unfiltered light is all gone and

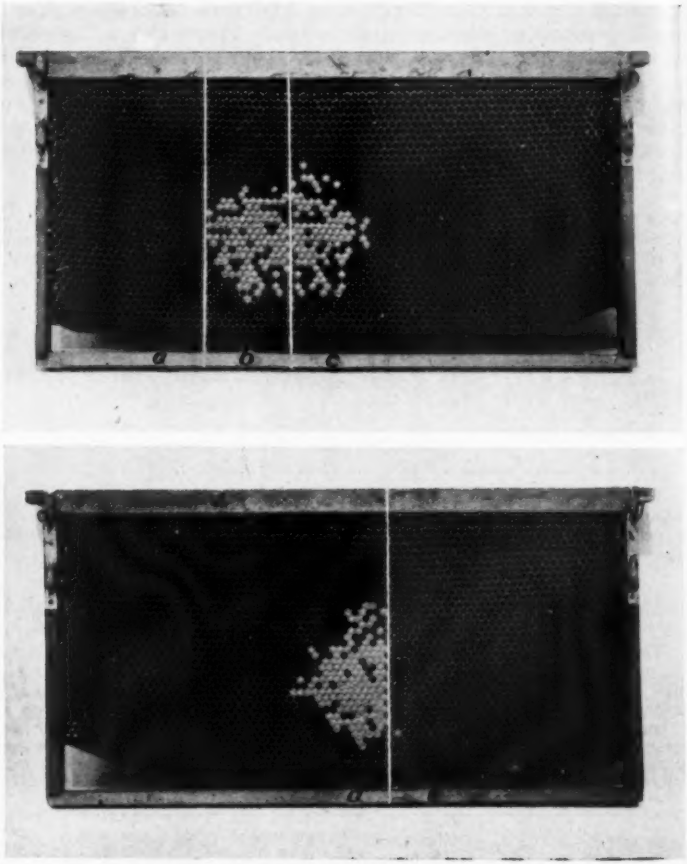

FIGURE 5.—Photographs of a frame of sealed brood in which different areas had been exposed to ultraviolet light when the larvae were 2 days old. The treatment of the various areas, lettered on the bottom bar of each frame, is given in table 11.

only one pupa remains in the area exposed to the light filtered through the Corex A glass. Since Corex A transmits rays down to 265 mμ wave length, whereas Corex D and Pyrex cut off the rays at 297 and 313 mμ, respectively, it is evident that it is the shorter rays that kill the bees. In this experiment, also, the irradiated bees did not emerge any sooner than the others.

The general result of the experiments, then, is that ultra-violet radiations of wave length shorter than 297 mμ given in the dosages used here have a decidedly lethal effect on honeybee larvae and exhibit no beneficial effects at all.

EFFECT OF ULTRAVIOLET RAYS ON QUEEN LARVAE

Perhaps the main interest in this research centers in the effect of ultraviolet rays on queens, both larvae and adults. The observation of Muth [12] that a certain queen, which had been irradiated while in the larval stage, was extraordinarily large leads one to think that here may be found a subject capable of being benefited by these rays. Also, it should be easy to note the effect of these rays on the length of the developmental period and on the weight of the adult queen when she emerges.

Two experiments were performed to test the effect of ultraviolet rays on the length of the developmental period and on the weight of the adult queen when she emerges. Day-old larvae were transferred to queen cups and given to a queenless colony. Most of them were accepted by the colony at once and given liberal quantities of royal jelly. The next day the hive was opened, the cups were removed, and those containing living larvae were divided into four groups: Group 1 was kept unexposed to the ultraviolet rays as a control, group 2 was exposed to the unfiltered light from the quartz mercury-vapor lamp, group 3 to the same light filtered through the Corex D filter, and group 4 to the same light filtered through the Pyrex filter. As shown in table 1, groups 2, 3, and 4 were thus exposed to radiations extending into the ultraviolet as far as 185, 297, and 313 mμ, respectively. In order to make the total energy received equal, these three groups were placed 72.6, 35.9, and 34.5 inches, respectively, from the lamp. In the first experiment the length of exposure on the first day was 5 seconds, and in the second experiment, 15 seconds. After the treatment the larvae were replaced in the hive. The next day they were given similar treatment except that the lengths of exposure were 15 and 45 seconds, respectively; they were then replaced in the hive as before. The following day the cells were sealed by the bees. Observations made from day to day showed whether or not the cells were still intact, an indication as to whether the baby queens inside were still alive. When it was nearly time for them to emerge, the queen cells were transferred to screen-wire cages such as are used by queen breeders, so that when a queen emerged she could not kill the queens within the other cells. By frequent examinations the time of emergence was ascertained fairly accurately. After emergence, in the second experiment, the queens were removed and weighed.

Table 12 shows that in both experiments the greatest mortality occurred when the larvae were irradiated with light from the unfiltered lamp; the next greatest mortality was among the larvae irradiated with the light filtered through the Corex D filter; while the least mortality was in experiment 1 among the controls and in experiment 2 among the larvae irradiated with light filtered through the Pyrex filter. Although the last-mentioned group seemed to receive some benefit from the light, the mortality among the controls was unusually high (33 percent) owing to unknown factors in the experiment, and hence this group probably received no actual benefit from the treatment.

[12] MUTH, C. F. See footnote 6.

TABLE 12.—*Effect of exposure of queen larvae 2 days old at the first treatment to the unfiltered light from a quartz mercury-vapor lamp, run at 4 amperes and 140 volts, and to two different spectral portions of it, at such distances that the total energy reaching the larvae was the same for all exposures*

Filter used	Distance from lamp	Exposed for 5 seconds on first day and 15 seconds on second day (experiment 1)							Exposed for 15 seconds on first day and 45 seconds on second day (experiment 2)							Average weight of adult queens
		Larvae treated	Larvae alive after indicated number of days			Adults emerged	Mortality	Length of developmental period [a]	Larvae treated	Larvae alive after indicated number of days			Adults emerged	Mortality	Length of developmental period [a]	
			1	4	5					1	2	4				
None, open lamp	*Inches* 31.5	*Number* 5	*Number* 5	*Number* 4	*Number* 3	*Number* 2	*Percent* 60	*Days* 16 (2)	*Number* 7	*Number* 6	*Number* 5	*Number* 3	*Number* 0	*Percent* 100	*Days*	*Milligrams*
Corex D	35.9	6	6	6	4	4	33	17 (2) 18 (2)	7	6	6	4	2	71	16 (1) 19 (1)	165
Pyrex	34.5	4	4	4	4	3	25	16 (3)	6	6	6	5	5	17	16 (2) 18 (1) 19 (2)	148
Controls (not irradiated)		7	7	7	7	6	14	16 (5) 17 (1)	6	6	4	4	4	33	16 (1) 18 (1) 19 (2)	174

[a] Numbers in parentheses refer to the number of adults having this developmental period.

No great reliance should be placed upon the exact percentages of mortality in these groups because of the small number of larvae composing them, but the results do seem to accord with those given previously in this paper and indicate that the shorter ultraviolet rays have a decidedly harmful effect on queen larvae.

The light apparently had no effect at all on the length of the developmental period (table 12), as the average period for the controls was no longer or shorter than that for the others. Considerable variation was noted, due probably in part to the fact that there was a difference of perhaps as much as a day between the age of the youngest and of the oldest larva selected at the beginning of the experiment.

Although too few queens were weighed to warrant the drawing of important conclusions as to the effect of irradiation on the weight of the adult queen, the average weight of both lots of irradiated queens which survived the treatment was less than that of the nonirradiated controls.

Here again in the treatment of queen larvae no benefit resulted from irradiation with ultraviolet light.

EFFECT OF IRRADIATION ON ADULT QUEENS

For several years queen bees irradiated with ultraviolet light have been sold in the United States, and also of late in England. The claims usually made in favor of this treatment are that it makes the queens more prolific and that the resulting progeny are more gentle—that is, less inclined to sting—than bees from nonirradiated queens. But when one attempts to test these claims he is faced with many difficulties. In the first place the gentleness of a colony cannot be measured accurately, and even if it could, it varies greatly among different races and strains of bees and sometimes even among sister colonies, being dependent on such factors as the weather, the condition of the honey flow, and the skill of the beekeeper. The rate of egg laying can be measured with some degree of accuracy, however, and observations can be made as to the vitality of such irradiated queens and the frequency of supersedure among them. With these problems in mind, the following experiment was performed.

One hundred queens were purchased from a queen breeder in the South. These were shipped to Washington in the ordinary Benton mailing cages, and all arrived in apparently good condition. From Washington they were taken by automobile 50 miles north to Westminster, Md., where they were at once divided into five groups of 20 queens each. Group 1 was treated with unfiltered light from the quartz mercury-vapor lamp, the cages being placed 72.6 inches from the lamp tube; group 2 was treated with the same light filtered through the Pyrex filter, at a distance of 34.5 inches; group 3 was treated with the same light filtered through the Corex D (clear) filter, at a distance of 35.9 inches; group 4 was treated with the same light filtered through the Corex A (red-purple) filter 15.7 inches from the lamp; and group 5 was left untreated as a control. Each of the first four groups was further divided into 10 pairs, and each pair was given a different length of exposure, from one sixth minute on each of the first three alternate days up to 10 minutes on the first and third days. Exposures were

made by placing the cages, wire-screen side toward the lamp, at the specified distance from the lamp tube for the specified length of time. Considerable heat was given out by the lamp, but the temperature at the cages, even after 10 minutes' exposure at 15.7 inches (using the Corex A filter), the shortest distance used, never rose above 30° C. (86° F.), which was below the temperature within the hive. Each day after the exposures were made the queen cages were hung in a special frame and placed in a hive of bees so that they might be kept at normal temperature and perhaps be given extra food by the workers. The cages containing the controls were hung in the same special frame and were thus taken from the hive every day along with those to be treated, and in all other respects were handled as nearly as possible like the cages containing the treated queens except that they were not exposed to the light.

Meanwhile, 100 2-pound packages of bees had been ordered and, through the cooperation of a commercial beekeeper, 100 hives had been prepared, each consisting of a single story with nine frames of empty drawn comb.

The treatments were administered between April 15 and 20, 1930. On April 23 the 2-pound packages arrived and were released into the hives, and with each of them was placed a queen cage containing a queen and several workers. In such a method of introducing queens the hole at one end of the cage is plugged with soft candy, which in the course of 2 to 3 days is eaten out by the workers in the hive, and the queen is thereby released.

Examination of the hives was then begun and continued for 3½ months, at first at intervals of approximately 1 week, but toward the last, of 2 weeks. Notice was taken particularly as to whether the original queen was still present in the hive or was being superseded by another. This could easily be ascertained, because before supersedure the bees build a series of queen cells and start rearing queens therein. Then, either during the development of these new queens in their queen cells or shortly before, the workers kill the old queen. Consequently, upon examining a hive in the process of supersedure, one usually finds several queen cells and at the same time notes a lack of eggs and very young larvae and the absence of the old queen.

Three times while the colonies were under observation measurements were made of the area of brood in the combs. The first measurement, after 43 days, was of sealed brood only, but in the other two, after 70 and 108 days, respectively, the entire brood was measured. This was done with a ruler; since the brood ordinarily occupies an elliptical area on each side of a comb, by measuring the length and breadth of this ellipse the area of the brood it contained could easily be computed.

The detailed results of this experiment are given in table 13, which shows the treatment given each of the 100 queens, whether or not she was alive at each examination both before and after introduction into the colonies, and, if she survived, gives the area of comb occupied by her brood at the time the different measurements were made.

TABLE 13.—*Record of the experiment to test the survival and rate of egg laying of queen bees simulated with*

TABLE 13.—*Record of the experiment to test the survival and rate of egg laying of queen bees irradiated with stated quantities of ultraviolet light, as compared with that of nonirradiated controls* [a]

GROUP 1. OPEN LAMP, 72.6 INCHES FROM LAMP

Queen no.	Length of exposure to light on indicated number of days after start of experiment							Condition of queens on indicated number of days [b]—													Area of brood on specified number of days after introduction		
								After start			After introduction													
	0	1	2	3	4	5	Total	6	7	8	7	15	21	28	35	43	52	70	84	96	108	43	70	108
	Min.	*Min.*	*Min.*	*Min.*	*Min.*	*Min.*	*Min.*															*Sq. in.*	*Sq. in.*	*Sq. in.*
1a	½	0	½	0	½	D	½																	
1b	½	0	½	0	½	0	½	A	A	*A* [c]	A	D												
2a	½	0	½	0	½	0	1½	A	A	*A*	A	A	A	A	D									
2b	½	0	½	0	½	0	1½	A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	425	725	605
3a	½	½	½	½	0	0	2	A	A	*A*	A	A	D											
3b	½	½	½	½	0	0	2	A	A	*A*	A	A	A	A	D									
4a	½	½	½	½	½	D	2½																	
4b	½	½	½	½	½	½	3	A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	195	610	425
5a	1	0	1	0	1	0	3	A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	475	955	480
5b	1	0	1	D			2																	
6a	1	0	2	0	D		3																	
6b	1	0	2	0	3	0	6	A	A	*A*	A	D												
7a	1	2	3	4	0	0	10	A	A	*A*	?	D												
7b	5	0	5	0	0	D	10																	
8a	5	0	5	0	0	0	10	A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	312	825	340
8b	5	0	5	0	0	D	10																	
9a	5	0	5	0	D		10																	
9b	5	0	5	0	D		10																	
10a	10	0	10	0	0	D	20																	
10b	10	0	10	0	0	0	20	A	A	*A*	D													

GROUP 2. PYREX FILTER, 34.5 INCHES FROM LAMP

11a	½	0	½	0	½	0	½	A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	445	810	590
11b	½	0	½	0	½	0	½	A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	340	795	470
12a	½	0	½	0	½	0	1½	A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	230	575	400
12b	½	0	½	0	½	0	1½	A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	(d)		
13a	½	½	½	½	0	0	2	A	A	*A*	D													
13b	½	½	½	½	0	0	2	A	A	*A*	A	A	A	A	A	A	D							
14a	½	½	½	½	½	½	3	A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	335	820	525
14b	½	½	½	½	½	½	3	A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	455	770	315
15a	1	0	1	0	1	0	3	A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	275	515	285

[a] The experiment was started on Apr. 15, and the queens were introduced into the colonies on Apr. 21.
[b] A signifies that this queen was alive, and D that she was dead on the day specified.
[c] Letters in this column are printed in italic to emphasize the fact that the queens were introduced into the colonies on this day.
[d] This queen laid drone eggs only.

TABLE 13.—*Record of the experiment to test the survival and rate of egg laying of queen bees irradiated with stated quantities of ultraviolet light, as compared with that of nonirradiated controls*—Continued

GROUP 2. PYREX FILTER, 34.5 INCHES FROM LAMP—Continued

Queen no.	Length of exposure to light on indicated number of days after start of experiment							Condition of queens on indicated number of days—													Area of brood on specified number of days after introduction		
								After start			After introduction													
	0	1	2	3	4	5	Total	6	7	8	7	15	21	28	35	43	52	70	84	96	108	43	70	108
	Min.	*Min.*	*Min.*	*Min.*	*Min.*	*Min.*	*Min.*															*Sq. in.*	*Sq. in.*	*Sq. in.*
15b	1	0	1	0	D	.....	2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
16a	1	0	2	0	3	0	6	A	A	*A*	A	A	A	A	A	A	D	.....	.....	.....	.....	610	.....	.....
16b	1	0	2	0	D	.....	3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
17a	1	2	3	4	0	0	10	A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	217	495	370
17b	1	2	3	4	0	0	10	A	A	*A*	A	A	A	A	D	.....	.....	.....	.....	.....	.....	.....	.....	.....
18a	5	0	5	0	0	D	10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
18b	5	0	5	0	0	0	10	A	A	*A*	A	A	A	A	D	.....	.....	.....	.....	.....	.....	.....	.....	.....
19a	5	0	5	0	5	0	15	A	A	*A*	A	A	A	A	A	A	A	D	.....	.....	.....	415	.....	.....
19b	5	0	5	0	5	0	15	A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	460	710	325
20a	10	0	10	0	D	.....	20	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
20b	10	0	10	D	.....	.....	20	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

GROUP 3. COREX D FILTER, 35.9 INCHES FROM LAMP

21a	1/6	0	1/6	0	1/6	D	½	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
21b	1/6	0	1/6	0	1/6	0	½	D	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
22a	½	0	½	0	½	0	1½	D	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
22b	½	0	½	0	½	0	1½	D	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
23a	½	½	½	½	0	0	2	A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	300	500	235
23b	½	½	½	½	D	.....	2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
24a	½	½	½	½	½	D	2½	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
24b	½	½	½	½	½	½	3	A	D	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
25a	1	0	D	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
25b	1	0	D	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
26a	1	0	D	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
26b	1	0	2	0	3	0	6	A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	340	655	445
27a	1	2	3	4	0	D	10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
27b	1	2	3	D	.....	.....	6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
28a	5	0	5	0	0	D	10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
28b	5	0	5	0	0	D	10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
29a	5	0	5	0	D	.....	10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
29b	5	0	D	.....	.....	.....	5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
30a	10	0	10	0	D	.....	20	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
30b	10	0	10	0	0	0	20	A	A	*A*	D	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

GROUP 4. COREX A FILTER, 15.7 INCHES FROM LAMP

GROUP 4. COREX A FILTER, 15.7 INCHES FROM LAMP

31a	1/16	0	1/16	0	1/16	0	1/2	A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	310	680	360
31b	1/16	0	1/16	0	1/16	0	1/2	A	A	*D*														
32a	1/2	0	1/2	0	1/2	0	1 1/2	A	A	*A*	A	A	A	A	D										
32b	1/2	0	1/2	0	1/2	0	1 1/2	A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	260	810	480
33a	1/2	1/2	1/2	1/2	0	0	2	A	A	*A*	A	A	A	A	D										
33b	1/2	1/2	1/2	1/2	0	0	2	D																	
34a	1/2	1/2	1/2	1/2	1/2	1/2	3	D																	
34b	1/2	1/2	1/2	1/2	1/2	1/2	3	D																	
35a	1	0	1	0	1	0	3	A	D																
35b	1	0	1	0	1	D	3																		
36a	1	0	2	0	3	D	6																		
36b	1	0	2	0	3	0	6	D																	
37a	1	2	3	4	0	D	10																		
37b	1	2	3	4	0	D	10																		
38a	5	0	5	0	0	0	10	D																	
38b	5	0	5	0	0	D	10																		
39a	5	0	5	0	5	0	15	A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	420	900	290
39b	5	0	5	0	5	0	15	A	D																
40a	10	0	10	0	0	0	20	D																	
40b	10	0	10	0	0	0	20	A	D																

GROUP 5. CONTROLS, UNTREATED

41								A*	A	*A*	A	A	A	A	D										
42								D																	
43								D																	
44								A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	230	605	335
45								A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	455	730	445
46								A	A	*D*															
47								D																	
48								A	A	*D*															
49								A	D																
50								D																	
51								D		*															
52								D																	
53								D																	
54								D																	
55								A	D																
56								A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	330	580	265
57								A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	200	630	320
58								A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	385	670	420
59								A	A	*A*	A	A	A	A	A	A	A	A	A	A	A	275	805	395
60								A	A	*A*	A	A	A	A	A	A	A	D				395		

* All control queens were alive 6 days after the start of the experiment.

SURVIVAL OF QUEENS

A large number of queens died during the treatment and before introduction into the colonies, only 42 surviving beyond this period. Such a high mortality need not always occur in an experiment of this kind, however. A number of unfavorable circumstances combined here to place the queens in a weakened physical condition—for example, a delay in delivery by the post, a ride of 50 miles by automobile on a chilly day, and inferior queen-cage candy that became too dry and hard toward the last, a condition which was not noticed in time to remedy it. Moreover, a subsequent test showed that under more favorable conditions queens may be kept in Benton cages for at least 2 weeks and during that time be given a total of as much as 1½ hours of irradiation and still survive apparently in good condition. But since in the present experiment the queens were treated as nearly alike as possible except for the irradiation and although weakened at the start were equally weakened, the results for the various groups are considered comparable.

When the number of survivals in the various groups (table 13) are compared, we find that the queens of group 2 survived far better than did those of any other group. These were the queens treated with light filtered through the Pyrex filter, and since Pyrex transmits only wave lengths of 313 mμ or longer, one may conclude that the screening out of the shorter rays saved many of the queens from death. As a matter of fact, a few more queens of group 2 survived than of the untreated controls (group 5), which indicates that the light filtered through Pyrex had either a beneficial effect or at least no harmful effect.

As a rule more queens died that received the longer treatments than those that received the shorter. For instance, not 1 of the 4 introduced queens that were exposed 20 minutes survived more than 1 week after introduction into the colony. As a matter of fact, if a comparison is made between all the queens receiving irradiation of 3 minutes or less and those exposed for 6 to 20 minutes, it will be found that 50 percent of the former survived until introduction and 62 percent of those introduced survived until the end of the experiment; whereas, of the latter, only 33 percent survived until introduction and only 39 percent of these survived until the end of the experiment.

A further result of this experiment, shown in table 13, is that up to the time of introduction about the same percentage of irradiated queens died as of nonirradiated controls, but that after introduction the supersedure was considerably greater among the irradiated queens. A count will show that of 30 irradiated queens introduced successfully (as evidenced by the fact that they were alive 1 week after introduction) to colonies, 12, or 40 percent, were superseded, while of the 8 nonirradiated queens introduced only 2, or 25 percent, were superseded. Some of the treated queens (nos. 25a, 25b, 26a, and 29b) died even before introduction, on the second day after the start of the experiment, whereas all the controls lived 6 days, indicating that the treatment had some harmful effect.

The net result of the experiment, therefore, is that irradiated queens have practically as good a chance of survival as nonirradiated ones kept during the same period, but that after introduction to colonies nonirradiated queens have a somewhat better chance of survival.

AREA OF BROOD

As to the area of brood produced by each queen, table 14 shows a slight advantage for the irradiated queens. For the 12 surviving queens which had received 3 minutes' or less irradiation (13 survived until the end of the experiment, but 1 of these produced only drone brood and was not included in these measurements), the average area of brood for the three examinations was 494 square inches and for the 5 which had received 6 to 20 minutes' irradiation, 474 square inches, making an average of 488 square inches for the 17 treated queens as compared with 449 square inches for the 6 untreated controls. From these data, if we disregard the errors inherent in this method of measuring the brood, it appears that the treated queens produced about 9 percent more brood than did the untreated queens. The same tendency was noted each time the area of brood was measured. On the forty-third day after introduction the advantage held by the irradiated queens was 9 percent, on the seventieth day about 7 percent, and on the one hundred and eighth day about 12 percent. Of the four groups of irradiated queens those of group 1 produced the most brood, although the number of queens surviving in most of the groups is too small to merit attaching much importance to the comparison.

As far as one may conclude from this experiment, then, the practice of irradiating queens with ultraviolet light is of no practical value to the beekeeper, for although it may result in a slightly greater rate of egg laying, there is also somewhat more supersedure among the queens during their first few months.

TABLE 14.—*Summary of data given in table 13, showing the average area of brood [a] produced by the queens receiving 3 minutes or less of treatment and those receiving from 6 to 20 minutes of treatment, together with the sum of the two groups as compared with the brood produced by queens receiving no treatment at all*

Group	Irradiated 3 minutes or less		Irradiated 6 to 20 minutes		Total for all irradiated queens		Nonirradiated queens	
	Number	Average area	Number	Average area	Number	Average area	Number	Average area
		Sq. in.		*Sq. in.*		*Sq. in.*		*Sq. in.*
1	3	544	1	492	4	531	----	----
2	6	497	2	430	8	480	----	----
3	1	345	1	480	2	413	----	----
4	2	483	1	537	3	501	----	----
Total or average	12	494	5	474	17	488	6	449

[a] The data given are averages of the measurements made on the forty-third, seventieth, and one hundred and eighth days after introduction.

SUMMARY AND CONCLUSIONS

The effect of irradiation with ultraviolet light on larvae and adults of both worker and queen honeybees was tested. Adult workers were irradiated in cages by exposing them to light from a quartz mercury-vapor lamp. The distance from the lamp, the length of exposure, and the wave length of the light were varied (the last by means of filters) in a great many combinations in the different tests. The effect of the irradiation was measured by ascertaining the average length of

life of the bees in each cage. The results showed that ultraviolet light, given in the larger dosages, is definitely harmful to bees. When the light was given in smaller quantities there was, in a few experiments, an apparently beneficial effect, but the results here were not consistent with those in other experiments; hence, the conclusion is that neither the total light from the lamp (185 to 1,400+mμ wave length) nor that portion of the light above 313 mμ, nor that portion above 297 mμ, nor that portion between 265 and 405 mμ has, in the dosages here used, any significant beneficial effect on the longevity of bees. Irradiation of the food (queen-cage candy) did not lengthen the average span of life.

Irradiation of worker larvae showed that they are easily harmed by ultraviolet rays, especially at the wave lengths shorter than 297 mμ, brood 3½ days old or younger being more susceptible than brood 4½ days of age or older. No beneficial effects were observed.

Similar results were obtained when queen larvae were irradiated. The effect of the shorter rays (below 297 mμ) proved decidedly harmful, while the longer rays had little or no effect. There was no shortening of the developmental period by any sort of irradiation used, and no significant difference in the weight of the emerging queens.

The irradiation of adult queens seemed to reduce their vitality somewhat, especially after the longer exposures, 6 to 20 minutes. The rate of egg laying of the irradiated queens was slightly greater, but any practical value to be derived from this is offset by the fact that there was considerably more supersedure among such queens.

The general harmful effect of the shorter ultraviolet rays, noted rather consistently in these experiments, together with the absence of any general good effect (except the slight increase in rate of egg laying by queens), makes it appear doubtful if irradiation of honeybee larvae or adults by ultraviolet light is commercially valuable.

EFFECT OF STAND IRREGULARITIES UPON THE ACRE YIELD AND PLANT VARIABILITY OF CORN [1]

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INTRODUCTION

The importance of extreme uniformity in the spacing of crop plants growing under field conditions is still a debatable question. An investigation of this factor in the production of corn (*Zea mays* L.) has been concluded at the Nebraska experiment station, and the results of 14 years' work are summarized in this paper. In a recent publication bearing upon this question Sprague and Farris [2] have cited a bibliography of 13 papers.

The extent to which variability in the development of individual corn plants is induced by stand irregularities is a phase of the problem not previously investigated. The procedure followed in the variability study was to measure large populations, grown under specified variable stand conditions, with respect to different characters, including (1) plant height at several stages of development, (2) ear height, (3) stalk diameter at first internode aboveground, (4) number of days from planting to silking, and (5) grain yield per ear-bearing plant. The standard deviations and coefficients of variation were calculated from these individual plant data.

Under farm conditions, stand irregularities associated with planting practices may result from such causes as imperfect germination, mechanical imperfections of the corn planter, and nonuniformity of seed size. According to tests conducted at the Nebraska station,[3] equally as good results may be expected whether the plants are individually spaced in rows, as in a drilled field, or whether they are grown in hills, as in a checked field. The stand which the corn grower strives to obtain is designed to occupy the land fully and to utilize all the field resources for crop growth. The usual aim is to have the plants equidistant in drills, or to have a constant number of plants in hills spaced at regular intervals.

KINDS OF STANDS COMPARED

In these studies the aim was to have an equal number of plants per acre, distributed both regularly and irregularly in hills 42 inches apart each way. The only variable factor in the different plantings under comparison was in the degree of irregularity of the stand. The variation in plant distribution was brought about by two methods, known herein as systematically variable and randomly variable planting. (1) In the experiment with systematically variable distri-

[1] Received for publication Mar. 31, 1933; issued October 1933. Contribution from the Department of Agronomy, Nebraska Agricultural Experiment Station, as paper no. 137 of the Journal Series.

[2] SPRAGUE, H. B., and FARRIS, N. F. THE EFFECT OF UNIFORMITY OF SPACING SEED ON THE DEVELOPMENT AND YIELD OF BARLEY. Jour. Amer. Soc. Agron. 23:516–533, illus. 1931.

[3] KIESSELBACH, T. A., ANDERSON, A., and LYNESS, W. E. TILLAGE PRACTICES IN RELATION TO CORN PRODUCTION. Nebr. Agr. Expt. Sta. Bul. 232:9. 1928.

bution, which was continued for 14 years, successive hills in the row were made to differ systematically in number of plants by planting sound seed of a standard local variety of dent corn at a double rate and thinning to the desired number. The following stands were compared: (*a*) Uniformly 3 plants per hill, (*b*) alternating 2- and 4-plant hills, (*c*) alternating 1-, 3-, and 5-plant hills, and (*d*) alternating 1-, 2-, 3-, 4-, and 5-plant hills. (2) In 1931 an additional comparison was made of randomly variable distributions in which the various stands were obtained by the use of four lots of seed having the respective germination percentages of 100, 75, 60, and 50. By planting these various samples at the rate of 3, 4, 5, and 6 seeds per hill, respectively, the number of plants per acre was approximately 10,668 in each instance, but the number of plants per hill was variable.

To obtain the specific germination desired, the seed samples were prepared by thoroughly mixing sound and dead seed in the necessary proportions. The dead seeds were identical with the sound seeds, except that their viability had been destroyed by heat for the purpose of this test.

Typical illustrations of stands obtained in both the systematically and randomly variable distributions are presented in figures 1 and 2. It does not appear that imperfections in any modern corn-planting device or nonuniformity in seed size could result in greater stand irregularities than have occurred here.

COMPARATIVE VARIABILITY OF STANDS

In the study of systematically variable distributions the plantings consisted of duplicate 4-row plots, 16 rods long, except in 1931 when there were six replications of 5-row plots 12 hills long. The plots of the randomly variable distributions were replicated 15 times and consisted of 4 rows 12 hills long. Border rows were discarded and the results were based on the interior rows.

In the case of the systematically variable stands it was especially difficult to visualize comparative variability; that is, to determine just how these stands rank as to nonuniformity, and how they compare with those obtained from adjusted planting rates with seed of various germination percentages. In order to appraise these differences the average deviations were calculated for all of the plant distributions, as shown in tables 1 and 2. From the data there presented it is evident that of the systematic distributions, the alternating 1-, 3-, and 5-plant hills give the least uniform stand and that an alternation of 1-, 2-, 3-, 4-, and 5-plant hills gives a more variable stand than is secured from an adjusted planting rate with seed having a germination as low as 50 percent.

TABLE 1.—*Average deviations from the mean number of plants per hill in the systematically variable plant distributions, 1915–17, 1920–29, and 1931*

Type of stand	Distribution of plants in successive hills	Mean	Average deviation
		Number	*Number*
Uniform	3-3-3-3	3	0.00
Uneven	2-4-2-4	3	1.00
Do	1-2-3-4-5	3	1.20
Do	1-3-5	3	1.33

UNIFORMLY 3-PLANT HILLS

3	3	3	3	3	3	3	3	3	3	3	3
3	3	3	3	3	3	3	3	3	3	3	3
3	3	3	3	3	3	3	3	3	3	3	3
3	3	3	3	3	3	3	3	3	3	3	3
3	3	3	3	3	3	3	3	3	3	3	3

ALTERNATING 2-AND 4-PLANT HILLS

2	4	2	4	2	4	2	4	2	4	2	4
4	2	4	2	4	2	4	2	4	2	4	2
2	4	2	4	2	4	2	4	2	4	2	4
4	2	4	2	4	2	4	2	4	2	4	2
2	4	2	4	2	4	2	4	2	4	2	4

ALTERNATING 1-3-AND 5-PLANT HILLS

1	3	5	1	3	5	1	3	5	1	3	5
3	5	1	3	5	1	3	5	1	3	5	1
5	1	3	5	1	3	5	1	3	5	1	3
1	3	5	1	3	5	1	3	5	1	3	5
3	5	1	3	5	1	3	5	1	3	5	1

ALTERNATING 1-2-3-4-AND 5-PLANT HILLS

1	2	3	4	5	1	2	3	4	5	1	2
2	3	4	5	1	2	3	4	5	1	2	3
3	4	5	1	2	3	4	5	1	2	3	4
4	5	1	2	3	4	5	1	2	3	4	5
5	1	2	3	4	5	1	2	3	4	5	1

FIGURE 1.—Diagram showing systematic distribution of hill types within 5-row plots. Numbers indicate the number of plants per hill.

PLOT NO. 4782. SEED GERMINATING 75 PERCENT DROPPED AT 4 KERNELS PER HILL

ROW 1	3	3	3	3	4	2	3	3	4	2	4	4
ROW 2	3	3	2	4	4	1	2	4	3	3	2	2
ROW 3	4	4	3	3	4	2	3	2	3	3	4	3
ROW 4	3	3	3	3	4	2	3	1	4	3	2	4

PLOT NO. 4783. SEED GERMINATING 60 PERCENT DROPPED AT 5 KERNELS PER HILL

ROW 1	3	3	4	4	2	2	3	5	5	4	4	4
ROW 2	2	3	3	1	3	4	2	4	4	2	4	2
ROW 3	4	3	3	4	3	2	2	3	3	2	2	3
ROW 4	4	5	2	1	4	3	3	5	4	4	2	4

PLOT NO. 4784. SEED GERMINATING 50 PERCENT DROPPED AT 6 KERNELS PER HILL

ROW 1	3	1	2	3	5	4	5	3	3	2	2	1
ROW 2	2	5	6	4	4	3	5	4	2	4	3	2
ROW 3	2	4	3	4	3	3	2	5	3	1	3	5
ROW 4	1	2	2	3	2	2	5	4	4	2	5	0

PLOT NO. 4785. SEED GERMINATING 100 PERCENT DROPPED AT 3 KERNELS PER HILL

ROW 1	3	3	3	3	3	3	3	3	3	3	3	3
ROW 2	3	3	3	3	3	3	3	3	3	3	3	3
ROW 3	3	3	3	3	3	3	3	3	3	3	3	3
ROW 4	3	3	3	3	3	3	3	3	3	3	3	3

FIGURE 2.—Diagram of four representative field plots, showing the number of plants in successive hills obtained from four lots of seed differing in germination percentage and planting rate. Numbers indicate the number of plants in each hill.

TABLE 2.—*Average deviations from the mean number of plants per hill in the randomly variable plant distributions, 1931*

Percentage germination of seed planted		Seeds dropped per hill	Mean	Average deviation
Laboratory	Field			
		Number	*Number*	*Number*
100	96.85	3	2.90	0.18
75	72.67	4	2.90	.73
60	58.50	5	2.91	.85
50	49.68	6	2.98	1.00

ACTUAL v. THEORETICAL STANDS FROM IMPERFECTLY GERMINATING SEED

Special interest attaches to the randomly variable stands in 1931 with respect to their approach in actual number of various hill types to the theoretically expected number. After proper technic had been employed to procure random distributions, seedling counts were made and recorded for each individual hill. Since the actual field germination and the number of seeds planted per hill were now known, it was possible by applying the law of chance to compute the frequency with which different hill types might be expected to occur in the various random distributions.

Under the conditions of this experiment four plant distributions were obtained from seed germinating 100, 75, 60, and 50 percent, planted 3, 4, 5, and 6 seeds per hill, respectively (table 2). The actual field germination based on seedling counts was 96.85, 72.67, 58.50, and 49.68 percent for the respective lots of seed. With these data, it was possible to determine the expected number of 0-, 1-, 2-, 3-, 4-, 5-, and 6-plant hills to be compared with the observed number. These data are presented in table 3. The formula employed in this comparison, and previously used by Kiesselbach and Ratcliff,[4] follows. The probability of dropping p sound kernels and q dead kernels from a composite sample containing m sound kernels and n dead kernels is:

$$\frac{m'}{(m-p)'} \quad \frac{(m+n-p-q)'}{(m+n)'} \quad \frac{n'}{(n-q)'} \quad \frac{(p+q)'}{p'\ q'}$$

where $m' = m(m-1)\ (m-2)\ (m-3)$----------$3.2.1$ = product of all integers from 1 to m.

If the χ^2 test for goodness of fit is applied to these data, the values of P are 0.4, 0.1, 0.8, and 0.2 for seed germinating nearly 100, 75, 60, and 50 percent, respectively. These values show that the deviations of the observed from the expected were not excessively large, and that imperfect germination and rate of planting account for the frequency distribution of the various hill types. In computing the foregoing values of P, the hill types were so grouped that no expected class contained fewer than five hills in accordance with the procedure suggested by Fisher.[5]

[4] KIESSELBACH, T. A., and RATCLIFF, J. A. FREEZING INJURY OF SEED CORN. Nebr. Agr. Expt. Sta. Research Bul. 16: 92. 1920.

[5] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 3, rev. and enl., 283 p., illus. Edinburgh and London. 1930.

TABLE 3.—*Frequency of various hill types resulting from seed germinating 100, 75, 60, and 50 percent,[a] planted at specified number of seeds per hill, 1931*

Plants per hill	Number of hills			Percentage of hills		
	Observed	Expected	Deviation	Observed	Expected	Deviation
FIELD GERMINATION 96.85 PERCENT AND 3 KERNELS DROPPED PER HILL						
0	1	0.02	0.98	0.139	0.003	0.136
1	8	2.05	5.95	1.113	.285	.828
2	50	63.83	−13.83	6.954	8.878	−1.924
3	660	653.10	6.90	91.794	90.834	.960
FIELD GERMINATION 72.67 PERCENT AND 4 KERNELS DROPPED PER HILL						
0	8	3.99	4.01	1.113	0.555	0.558
1	51	42.59	8.41	7.093	5.923	1.170
2	157	170.22	−13.22	21.836	23.674	−1.838
3	288	301.83	−13.83	40.055	41.980	−1.925
4	215	200.37	14.63	29.903	27.868	2.035
FIELD GERMINATION 58.50 PERCENT AND 5 KERNELS DROPPED PER HILL						
0	7	8.76	−1.76	0.979	1.225	−0.246
1	65	61.93	3.07	9.091	8.661	.430
2	174	174.90	−.90	24.336	24.462	−.126
3	257	246.71	10.29	35.944	34.505	1.439
4	161	173.79	−12.79	22.517	24.306	−1.789
5	51	48.91	2.09	7.133	6.841	.292
FIELD GERMINATION 49.68 PERCENT AND 6 KERNELS DROPPED PER HILL						
0	18	11.64	6.36	2.503	1.619	0.884
1	70	69.10	.90	9.736	9.611	.125
2	172	170.73	1.27	23.922	23.746	.176
3	207	224.81	−17.81	28.790	31.267	−2.477
4	162	166.36	−4.36	22.531	23.137	−.606
5	79	65.60	13.40	10.988	9.123	1.865
6	11	10.76	.24	1.530	1.497	.033

[a] Field germination was slightly less than the designated percentages.

EFFECT OF UNEVEN STANDS UPON THE ACRE-YIELD OF GRAIN

SYSTEMATICALLY VARIABLE STANDS

The annual and average yields secured during 14 years from three systematically variable plant distributions where the number of plants per acre has remained constant and the number of plants per hill has varied systematically, are reported in table 4 in comparison with a uniform stand. The average of the three uneven distributions was 50 bushels as compared with 49.9 bushels for uniformly 3-plant hills. Under the conditions of these extreme variations in stand the actual yield has not been significantly affected.

A summary of yield and various other agronomic characters is given in table 5. It is evident that these inequalities of stand have not, to any important degree, affected the general development of the crop.

TABLE 4.—*Effect of systematically variable plant distribution upon the yield in bushels per acre of corn, 1915–17, 1920–29, and 1931*[a]

Year	Yield per acre from plants in successive hills (10,668 plants per acre) in—				
	Variable stands				Uniformly 3-plant stands
	Alternating 2 and 4 plants	Alternating 1, 3, and 5 plants	Alternating 1, 2, 3, 4, and 5 plants	Average for uneven stands	
1915	89.8	88.0	95.1	91.0	93.4
1916	58.4	52.4	54.4	55.1	55.2
1917	28.1	26.3	30.6	28.3	26.7
1920	51.9	48.9	50.2	50.3	52.3
1921	68.0	64.3	62.9	65.1	66.7
1922	45.1	45.9	45.4	45.5	44.7
1923	69.8	65.0	65.4	66.7	70.9
1924	43.9	43.2	44.1	43.7	40.9
1925	42.9	43.0	41.9	42.6	39.2
1926	24.7	23.4	23.2	23.8	22.4
1927	52.2	53.1	53.9	53.1	53.1
1928	47.6	50.8	46.1	48.2	44.1
1929	60.3	60.9	61.1	60.8	64.2
1931	26.3	24.3	25.8	25.5	24.2
Average	50.6	49.3	50.0	50.0	49.9

[a] Tests made in duplicate 4-row plots 16 rods long, except in 1931, when there were 6 replications of 5-row plots, 12 hills long. Yields are calculated on the basis of shelled corn containing 14 percent moisture.

TABLE 5.—*Effect of systematically variable distribution of corn plants upon various agronomic characters, during 14 years,*[a] *1915–17, 1920–29, and 1931*

[In tassel, Aug. 3; ripe, Sept. 22]

Type of stand	Distribution of plants in successive hills	Height		Per 100 plants					Shrinkage	Shelling	Yield per acre
		Stalk	Ear	Smutted	Barren	2-eared	Suckers	Lodged			
		Inches	*Inches*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*	*Percent*	*Bushels*
Uneven	2-4-2-4	93.7	45.9	7.9	6.8	8.6	16.4	16.7	7.4	82.7	50.6
Do	1-3-5	94.4	44.5	7.8	7.9	8.9	18.3	14.8	6.7	82.9	49.3
Do	1-2-3-4-5	93.8	44.7	8.8	8.6	9.7	21.0	13.7	6.9	83.4	50.0
Average		94.0	45.0	8.2	7.8	9.1	18.6	15.1	7.0	83.0	50.0
Uniform	3-3-3-3	94.6	45.5	8.5	6.8	8.4	15.1	15.8	7.4	82.8	49.9

[a] Data on vegetative characters are averages for 10 years only.

RANDOMLY VARIABLE STANDS

For the crop grown in 1931 from lots of seed germinating 100, 75, 60, and 50 percent planted at the rate of 3, 4, 5, and 6 seeds per hill, respectively, the corresponding yields (table 6) were 24.96 ± 0.65, 25.50 ± 0.66, 25.34 ± 0.66, and 25.12 ± 0.65 bushels per acre. Any differences in yield are within the probable error, and it is concluded that these variable stands have not affected acre yields. Other agronomic characteristics of the crop have been so little changed as to be of no very material consequence.

TABLE 6.—*Effect of randomly variable distribution of corn plants upon various agronomic characters and yield, 1931* [a]

Percentage germination of seed planted	Seeds dropped per hill	Per 100 plants				Yield per acre
		Barren	2-eared stalks	Suckers	Broken or lodged	
	Number	*Number*	*Number*	*Number*	*Number*	*Bushels*
75	4	26.6	3.1	6.3	0.9	25.50±0.66
60	5	22.4	3.4	5.6	.8	25.34± .66
50	6	27.1	2.5	4.4	.9	25.12± .65
Average		25.4	3.0	5.4	.9	25.32± .38
100	3	22.4	2.4	4.5	.7	24.96± .65

[a] Averages for 15 replications of 4-row plots 12 hills long. Yields are calculated on the basis of shelled corn containing 14 percent moisture.

EFFECT OF UNEVENNESS OF STAND UPON THE DEVELOPMENT AND VARIABILITY OF CORN PLANTS

CALCULATION OF BIOMETRICAL CONSTANTS

The influence of stand irregularities upon different plant characters as measured by the standard deviation and coefficient of variation was determined in 1931. These constants are reported in the succeeding variability tables, together with the mean value for the character concerned.

Differences between the constants for the variable and the uniform stands are reported in the lower part of each table. These differences will suggest how much more or less variable are the uneven than the even stands. Statistical significance is expressed in the tables only by the D/PE (the difference divided by the probable error of the difference) calculations. In the analysis of these data statistical significance is attached to any difference which is approximately three times its probable error. When the chances of exceeding a given difference in one direction only are considered, this is equivalent to odds of about 39:1, or if the chances of exceeding a given difference in either direction are considered, the odds are 19:1.

The formulas used in calculating the various constants are as follows:

(1) Standard deviation =

$$\sqrt{\frac{Sfd^2}{n}-\frac{S(fd)^2}{n^2}-c/12}$$

where n = number of variates, fd = frequency × deviation from arbitrary origin, and $c/12$ = Sheppard's correction for grouping according to Ezekiel.[6]

(2) Combined standard deviation =

$$\sqrt{\frac{S_1(x_1-\bar{x}_1)^2+S_2(x_2-\bar{x}_2)^2}{(N_1-1)+(N_2-1)}}$$

where $S_1(x_1-\bar{x}_1)^2$ = sum of squares of the deviations from the sample mean and N_1 = number of variates.[7]

All other statistical constants employed were computed in accordance with standard, authoritative procedure.

[6] EZEKIEL, M. METHODS OF CORRELATION ANALYSIS. 427 p., illus. New York and London. 1930.
[7] TIPPETT, L. H. C. THE METHODS OF STATISTICS; AN INTRODUCTION MAINLY FOR WORKERS IN THE BIOLOGICAL SCIENCES. 222 p., illus. London. 1931.

MATURE PLANT HEIGHT

At maturity (tables 7 and 8) plant-height measurements were taken in inches from the ground level to the lowest tassel branch. In all cases, with both types of variable distributions, mean plant height has been depressed a fraction of an inch. The average reduction was 0.81 inch in the case of the systematic and 0.56 inch in the case of the randomly variable distributions. These differences have D/PE values of 3.38 and 3.11, respectively, and are suggestive of reduction in mean plant height. The differences are so small, however, as to have no agronomic importance.

TABLE 7.—*Effect of systematically variable plant distribution upon the plant height of corn and its variability at maturity, 1931*

CONSTANTS FOR THE VARIOUS PLANT DISTRIBUTIONS

Type of stand	Distribution of plants in successive hills	Plants measured	Height of plants		
			Mean	Standard deviation	Coefficient of variation
		Number	*Inches*	*Inches*	
Uneven	2-4-2-4	627	69.50	8.01	11.52
Do	1-3-5	624	69.70	8.27	11.86
Do	1-2-3-4-5	638	69.47	8.54	12.29
Average [a]			69.56	8.29	11.92
Uniform	3-3-3-3	633	70.37	7.29	10.36

DEVIATION OF PLANT CONSTANTS IN THE UNEVEN STANDS FROM THOSE IN THE UNIFORM STAND

Distribution of plants in successive hills	Height of plants					
	Mean		Standard deviation		Coefficient of variation	
	Difference	D/PE	Difference	D/PE	Difference	D/PE
	Inches		*Inches*		*Inches*	
2-4-2-4	−0.87	2.90	0.72	3.60	1.16	3.87
1-3-5	−.67	2.23	.98	4.67	1.50	5.00
1-2-3-4-5	−.90	3.00	1.25	5.95	1.93	6.22
Average [b]	−.81	3.38	1.00	5.88	1.56	6.50

[a] For the averages, a weighted mean and combined standard deviation have been calculated and the coefficient of variation was derived from them.

[b] The average differences were found by comparing the weighted mean, combined standard deviation, and coefficient of variation with the corresponding constants of the normal distribution.

TABLE 8.—*Effect of randomly variable plant distribution upon the plant height of corn and its variability at maturity, 1931*

CONSTANTS FOR THE VARIOUS PLANT DISTRIBUTIONS

Percentage germination of seed planted	Seeds dropped per hill	Plants measured	Height of plants		
			Mean	Standard deviation	Coefficient of variation
	Number	*Number*	*Inches*	*Inches*	
75	4	1,013	66.75	8.12	12.16
60	5	960	67.52	7.88	11.67
50	6	1,052	66.76	8.06	12.07
Average [a]			67.00	8.03	11.98
100	3	999	67.56	7.10	10.51

TABLE 8.—*Effect of randomly variable plant distribution upon the plant height of corn and its variability at maturity, 1931*—Continued

DEVIATION OF PLANT CONSTANTS IN THE UNEVEN STANDS FROM THOSE IN THE NORMAL STAND

Percentage germination of seed planted	Height of plants					
	Mean		Standard deviation		Coefficient of variation	
	Difference	D/PE	Difference	D/PE	Difference	D/PE
	Inches		*Inches*		*Inches*	
75	−0.81	3.52	1.02	6.38	1.65	6.88
60	−.04	.17	.78	4.88	1.16	4.83
50	−.80	3.48	.96	6.00	1.56	6.50
Average *	−.56	3.11	.93	7.15	1.47	7.74

* Refer to table 7, footnotes.

Variability in plant height, as indicated by the standard deviation and coefficient of variation, was slightly greater in the variable distributions. The combined standard deviations for the variable stands of both types of planting were significantly larger than the standard deviations for the uniform stands. The increases were 1 inch for the systematic and 0.93 inch for the random distributions. The coefficients of variation corroborate the standard deviations in this respect.

While mature plant height was slightly reduced and variability increased because of a lack of uniformity in stands, the differences were too small to have much agronomic importance.

PLANT HEIGHT AT IMMATURE STAGES

Plant-height measurements were taken on all the plants in the systematically variable distributions 7 and 9 weeks after planting. Similar records were made for the random distributions 4 and 7 weeks after planting. Measurements were taken to the maximum height when the leaves were held erect and are designated as "stretched height". For the sake of brevity, since no material differences were found, the detailed results are not given in table form.

Seven weeks after planting, when the stretched height was approximately 48 inches, the mean height of the plants grown in systematically variable distributions was 0.18 ± 0.18 inch shorter; the combined standard deviation 0.12 ± 0.12 inch less, and the coefficient of variation 0.21 ± 0.26 percent less than for plants grown in uniformly 3-plant hills. Nine weeks after planting, when the stretched height of plants was about 72 inches, the mean height was 0.56 ± 0.22 inch shorter; the combined standard deviation 0.43 ± 0.15 inch larger, and the coefficient of variation 0.67 ± 0.22 percent larger as an average for all three systematically variable distributions than for plants grown in uniformly 3-plant hills. These differences approach significance, but are too slight to have importance.

For plants resulting from seed germinating 100, 75, 60, and 50 percent planted at compensating rates, the mean height (stretched) of approximately 36 inches 4 weeks after planting was 0.27 ± 0.09 inch less; the combined standard deviation 0.58 ± 0.07 inch larger, and the coefficient of variation 1.72 ± 0.19 percent larger for plants

grown from imperfectly germinating seed than for those resulting from seed germinating 100 percent. At 7 weeks after planting, when stretched height was about 61 inches, the mean height was 0.81 ± 0.14 inch shorter; the combined standard deviation 0.39 ± 0.09 inch larger, and the coefficient of variation 0.77 ± 0.16 percent larger for plants resulting from imperfectly germinating seed than for those resulting from seed germinating 100 percent. These differences are statistically significant but not important agronomically.

EAR HEIGHT

The height of ears was measured from the ground level to the ear node of the stalk. There was an average increase of 0.18 inch for mean ear height in case of the systematic and a decrease of 0.10 inch in case of the randomly variable stands. These differences (tables 9 and 10) have D/PE values of 1 and 0.67, respectively, and are not statistically significant.

The combined standard deviations and coefficients of variation for the variable stands were not significantly different from those for the uniform stands. Thus no important ear-height effects resulted from these stand irregularities.

TABLE 9.—*Effect of systematically variable plant distribution upon the ear height of corn and its variability, 1931*

CONSTANTS FOR THE VARIOUS PLANT DISTRIBUTIONS

Type of stand	Distribution of plants in successive hills	Plants measured	Ear height		
			Mean	Standard deviation	Coefficient of variation
		Number	*Inches*	*Inches*	
Uneven	2-4-2-4	610	37.08	5.78	15.59
Do	1-3-5	599	37.95	5.84	15.39
Do	1-2-3-4-5	621	37.35	6.26	16.76
Average [a]			37.46	5.97	15.94
Uniform	3-3-3-3	615	37.28	5.79	15.53

DEVIATION OF PLANT CONSTANTS IN THE UNEVEN STANDS FROM THOSE IN THE UNIFORM STAND

Distribution of plants in successive hills	Ear height					
	Mean		Standard deviation		Coefficient of variation	
	Difference	D/PE	Difference	D/PE	Difference	D/PE
	Inches		*Inches*			
2-4-2-4	−0.20	0.87	−0.01	0.06	0.06	0.14
1-3-5	.67	2.91	.05	.31	−.14	.32
1-2-3-4-5	.07	.30	.47	2.94	1.23	2.80
Average [a]	.18	1.00	.18	1.38	.41	1.17

[a] Refer to table 7, footnotes.

TABLE 10.—*Effect of randomly variable plant distribution upon the ear height of corn and its variability at maturity, 1931*

CONSTANTS FOR THE VARIOUS PLANT DISTRIBUTIONS

Percentage germination of seed planted	Seeds dropped per hill	Plants measured	Ear height		
			Mean	Standard deviation	Coefficient of variation
	Number	*Number*	*Inches*	*Inches*	
75	4	949	40.29	5.75	14.27
60	5	912	40.17	6.10	15.18
50	6	919	40.05	5.54	13.83
Average [a]			40.17	5.80	14.44
100	3	934	40.27	5.72	14.20

DEVIATION OF PLANT CONSTANTS IN THE UNEVEN STANDS FROM THOSE IN THE NORMAL STAND

Percentage germination of seed planted	Ear height					
	Mean		Standard deviation		Coefficient of variation	
	Difference	D/PE	Difference	D/PE	Difference	D/PE
	Inches		*Inches*			
75	0.02	0.11	0.03	0.23	0.07	0.22
60	−.10	.53	.38	2.92	.98	2.97
50	−.22	1.22	−.18	1.38	−.37	1.16
Average [a]	−.10	.67	.08	.80	.24	.92

[a] Refer to table 7, footnotes.

STALK DIAMETER

Stalk-diameter determinations were made only upon the plants in the randomly variable distributions, or the plants resulting from seed corn with imperfect germination. This character, apparently, was definitely affected by uneven plant distribution. The results are presented in table 11.

The mean stalk diameter for plants grown from seed germinating 100 percent was 23.72 mm, while for plants in the variable distributions, grown from imperfectly germinating seed, the average mean diameter was 23.14 mm. These values show a decrease of 0.58 mm in stalk diameter for plants of the irregular stands. In every instance mean stalk diameter was smaller for plants grown in the uneven distributions.

Variability of stalk diameter was increased in the uneven plant distributions. Under normal stand conditions the standard deviation was 2.54 mm, while the combined standard deviation for the variable stands was 3.11 mm. This corresponds to an increase of 0.57 mm for the irregular stands. The average coefficient of variation for plants grown in variable distributions was 2.73 percent larger than for the normal distribution. From these data it may be concluded that mean stalk diameter was 2.4 percent smaller while variability was significantly greater in the uneven stands.

TABLE 11.—*Effect of randomly variable plant distribution upon the stalk diameter of corn and its variability at maturity, 1931*

CONSTANTS FOR THE VARIOUS PLANT DISTRIBUTIONS

Percentage germination of seed planted	Seeds dropped per hill	Plants measured	Stalk diameter		
			Mean	Standard deviation	Coefficient of variation
	Number	*Number*	*Millimeters*	*Millimeters*	
75	4	1,024	23.23	2.94	12.66
60	5	968	23.46	3.20	13.64
50	6	1,042	22.74	3.16	13.90
Average [a]			23.14	3.11	13.44
100	3	1,001	23.72	2.54	10.71

DEVIATION OF PLANT CONSTANTS IN THE UNEVEN STANDS FROM THOSE IN THE NORMAL STAND

Percentage germination of seed planted	Stalk diameter					
	Mean		Standard deviation		Coefficient of variation	
	Difference	D/PE	Difference	D/PE	Difference	D/PE
	Millimeters		*Millimeters*			
75	−0.49	6.12	0.40	6.67	1.95	7.80
60	−.26	2.89	.66	11.00	2.93	11.27
50	−.98	10.89	.62	10.33	3.19	12.27
Average [a]	−.58	9.67	.57	11.40	2.73	13.65

[a] Refer to table 7, footnotes.

MATURITY

It is generally recognized that date of silking may be used as a measure of the relative time of maturity in corn. Silking notes were taken only on the plants grown in connection with the randomly variable stands. The data from this test are shown in table 12. In no instance was the number of days from planting to silking affected by plant distribution. The mean for plants resulting from seed germinating 100 percent was 68.68 days, while for plants grown in the variable distributions it was 68.79 days. In no case was there a significant increase or decrease in mean silking date or its variability.

TABLE 12.—*Effect of randomly variable plant distribution upon the length of interval from planting to silking and its variability, 1931*

CONSTANTS FOR THE VARIOUS PLANT DISTRIBUTIONS

Percentage germination of seed planted	Seeds dropped per hill	Plants measured	Length of interval		
			Mean	Standard deviation	Coefficient of variation
	Number	*Number*	*Days*	*Days*	
75	4	926	68.72	3.78	5.50
60	5	914	68.55	3.74	5.46
50	6	904	69.11	3.80	5.50
Average [a]			68.79	3.78	5.49
100	3	924	68.68	3.65	5.31

[a] Refer to table 7, footnotes.

TABLE 12.—*Effect of randomly variable plant distribution upon the length of interval from planting to silking and its variability, 1931*—Continued

DEVIATION OF PLANT CONSTANTS IN THE UNEVEN STANDS FROM THOSE IN THE NORMAL STAND

Percentage germination of seed planted	Length of interval					
	Mean		Standard deviation		Coefficient of variation	
	Difference	D/PE	Difference	D/PE	Difference	D/PE
	Days		*Days*			
75	0.04	0.36	0.13	1.62	0.19	1.58
60	−.13	1.18	.09	1.12	.15	1.25
80	.43	3.91	.15	1.88	.19	1.58
Average *	.11	1.22	.13	1.86	.18	2.00

* Refer to table 7, footnotes.

GRAIN YIELD PER EAR-BEARING PLANT

Determinations of shelled-corn weight per ear-bearing plant for the systematically uneven distributions are reported in table 13. In no instance was there a significant difference in the means, although it is observed that they were slightly higher for the uneven than for the uniform stand. Uneven distribution, however, increased variability, as is indicated by an increase of 6.28 g in the combined standard deviation for uneven stands above the standard deviation for plants grown in uniformly 3-plant hills. However, the coefficient of variation, which was 5.15 percent greater for the uneven distributions, is not so suggestive of increased variability. Thus, according to the standard deviation, the increase in variability was statistically significant, whereas judged by the coefficient of variation it was not significant.

Table 14 presents the data for ears resulting from plants grown in the randomly variable distributions. As in the preceding data, significant differences in means were not found. In this instance, however, no induced variability was indicated by either the standard deviation or the coefficient of variation.

TABLE 13.—*Effect of systematically variable plant distribution upon the grain yield (moisture-free) of ear-bearing plants and its variability, 1931*

CONSTANTS FOR THE VARIOUS PLANT DISTRIBUTIONS

Type of stand	Distribution of plants in successive hills	Plants measured	Grain yield per plant		
			Mean	Standard deviation	Coefficient of variation
		Number	*Grams*	*Grams*	
Uneven	2-4-2-4	490	64.63	48.87	75.62
Do	1-3-5	487	68.74	52.43	76.27
Do	1-2-3-4-5	504	67.88	51.77	76.27
Average *			67.09	50.98	75.99
Uniform	3-3-3-3	500	63.10	44.70	70.84

* Refer to table 7, footnotes.

TABLE 13.—*Effect of systematically variable plant distribution upon the grain yield (moisture-free) of ear-bearing plants and its variability, 1931*—Continued

DEVIATION OF PLANT CONSTANTS IN THE UNEVEN STANDS FROM THOSE IN THE UNIFORM STAND

Distribution of plants in successive hills	Grain yield per plant					
	Mean		Standard deviation		Coefficient of variation	
	Difference	D/PE	Difference	D/PE	Difference	D/PE
	Grams		*Grams*			
2-4-2-4	1.53	0.76	4.17	2.94	4.78	1.49
1-3-5	5.64	2.70	7.73	5.22	5.43	1.68
1-2-3-4-5	4.78	2.32	7.07	4.88	5.43	1.70
Average *	3.99	2.46	6.28	5.51	5.15	2.02

* Refer to table 7, footnotes.

TABLE 14.—*Effect of randomly variable plant distribution upon the grain yield (moisture-free) of ear-bearing plants and its variability, 1931*

CONSTANTS FOR THE VARIOUS PLANT DISTRIBUTIONS

Percentage germination of seed planted	Seeds dropped per hill	Plants measured	Grain yield per plant		
			Mean	Standard deviation	Coefficient of variation
	Number	*Number*	*Grams*	*Grams*	
75	4	749	76.36	46.77	61.25
60	5	748	75.58	46.62	61.68
50	6	765	74.11	46.18	62.31
Average *			75.34	46.57	61.81
100	3	748	73.44	44.82	61.03

DEVIATION OF PLANT CONSTANTS IN THE UNEVEN STANDS FROM THOSE IN THE NORMAL STAND

Percentage germination of seed planted	Grain yield per plant					
	Mean		Standard deviation		Coefficient of variation	
	Difference	D/PE	Difference	D/PE	Difference	D/PE
	Grams		*Grams*			
75	2.92	1.84	1.95	1.72	0.22	0.11
60	2.14	1.34	1.80	1.61	.65	.32
50	.67	.43	1.36	1.21	1.28	.64
Average *	1.90	1.48	1.75	1.92	.78	.48

* Refer to table 7, footnotes.

PERFORMANCE OF PLANTS IN THE VARIOUS HILL TYPES

While the standard deviation is an absolute measure and the coefficient of variation a relative measure of variability, these constants do not fully reveal plant differences induced by the specific hill type.

Some interest attaches to an analysis of the plant development within the various kinds of hills classified according to the number of plants contained. It would seem that differences in the development and performance of plants growing in the various hill types may be attributed directly to the various degrees of plant competition within the hill resulting from stand inequalities. The results from the variable plant distribution studies in 1931 are summarized in this manner in tables 15 and 16.

TABLE 15.—*Performance of plants within each hill type of the systematically variable plant distributions, 1931*

Hill type (plants per hill)	Percentages of all plants, all ear-bearing plants, and all barren plants, that occurred in each hill type			Performance of plants within each hill type			
	Total plants	Ear-bearing plants	Barren plants	Mature plant height	Barren plants	Shelled corn *	
						Per plant	Per ear-bearing plant
				Inches	*Percent*	*Grams*	*Grams*
UNIFORMLY 3 PLANTS							
3	100.0	100.0	100.0	70.4	18.6	51.3	63.1
ALTERNATING 2 AND 4 PLANTS							
2	33.3	37.0	19.2	71.2	12.0	71.1	80.7
4	66.7	63.0	80.8	68.5	25.1	41.5	55.4
Mean				69.5	20.7	51.4	64.6
ALTERNATING 1, 3, AND 5 PLANTS							
1	11.1	12.8	2.0	75.4	2.9	121.0	124.6
3	33.3	33.3	33.3	70.6	16.4	61.7	73.8
5	55.6	53.8	64.7	68.0	19.0	41.1	50.8
Mean				69.7	16.4	56.8	68.7
ALTERNATING 1, 2, 3, 4, AND 5 PLANTS							
1	6.7	7.8	0.9	74.8	2.4	114.4	117.2
2	13.3	14.7	7.1	75.4	9.4	89.4	98.7
3	20.0	21.3	14.1	70.8	12.5	66.7	76.2
4	26.7	26.1	29.2	67.1	19.4	39.9	49.5
5	33.3	30.1	48.7	67.0	25.8	37.3	50.3
Mean				69.5	17.7	56.0	67.9

* Moisture-free.

In the case of the three different systematically variable stands (1) with alternating 2- and 4-plant hills, 33.3 and 66.7 percent of the plant population occurred in the respective hill types; (2) with alternating 1-, 3-, and 5-plant hills, 11.1, 33.3, and 55.6 percent of the plants were grown in the respective hill types; and (3) with alternating 1-, 2-, 3-, 4-, and 5-plant hills, 6.7, 13.3, 20.0, 26.7, and 33.3 percent of the plants occurred, respectively, in the various hill types.

TABLE 16.—*Effect of randomly variable distribution of corn plants upon the performance of plants within each hill type of the various distributions, 1931*

Hill type (plants per hill)	Percentages of all plants, all ear-bearing plants, and all barren plants, that occurred in each hill type			Performance of plants within each hill type			
						Shelled corn [a]	
	Total plants	Ear-bearing plants	Barren plants	Mature plant height	Barren plants	Per plant	Per ear-bearing plant
				Inches	*Percent*	*Grams*	*Grams*
GERMINATING 100 PERCENT AND 3 KERNELS DROPPED PER HILL							
1	0.4	0.5	0	74.2	0	115.0	115.0
2	4.8	4.3	6.1	68.0	29.2	67.4	95.2
3	94.8	95.2	93.9	67.0	21.7	57.0	72.8
Mean				67.6	22.4	57.7	73.4
GERMINATING 75 PERCENT AND 4 KERNELS DROPPED PER HILL							
1	2.4	2.6	1.9	70.8	20.0	84.7	105.8
2	15.0	15.9	12.3	69.7	20.6	79.6	100.3
3	41.4	41.4	41.4	66.6	25.2	57.0	76.2
4	41.2	40.1	44.4	64.8	27.2	46.2	63.5
Mean				66.8	26.6	56.6	76.4
GERMINATING 60 PERCENT AND 5 KERNELS DROPPED PER HILL							
1	3.1	4.0	0	71.1	0	118.9	118.7
2	16.7	19.1	8.0	69.4	10.2	78.7	87.6
3	37.0	35.2	43.7	67.7	25.3	58.0	77.9
4	30.9	30.0	34.3	65.8	23.8	48.1	63.1
5	12.2	11.8	14.0	62.2	24.6	49.2	65.3
Mean				67.5	22.4	59.2	75.6
GERMINATING 50 PERCENT AND 6 KERNELS DROPPED PER HILL							
1	3.3	4.2	0.4	71.0	2.9	103.9	107.0
2	16.0	19.0	7.3	69.2	11.6	88.0	99.5
3	29.0	29.0	29.1	67.2	25.6	54.0	72.6
4	30.2	27.7	37.4	66.3	31.6	45.0	65.8
5	18.4	17.9	20.0	62.5	27.6	45.5	62.9
6	3.1	2.1	5.8	63.8	48.5	24.7	48.0
Mean				66.7	27.1	55.9	74.1

[a] Moisture-free.

In the case of randomly variable stands resulting from the use of imperfectly germinating seed planted at an increased rate to compensate for dead seeds, the resulting 1-, 2-, and 3-plant hills from seed germinating 100 percent contained 0.4, 4.8, and 94.8 percent of the plant population, respectively. When seed corn germinating 75 percent was planted at 4 kernels per hill, 2.4, 15.0, 41.4, and 41.2 percent of the plants grew in 1-, 2-, 3-, and 4-plant hills, respectively. For seed corn germinating 60 percent and planted at 5 kernels per hill, the resulting percentages of the population occurring in 1-, 2-, 3-, 4-, and 5-plant hills were 3.1, 16.7, 37.0, 30.9, and 12.2 percent, respectively. And seed corn germinating 50 percent, planted at 6

kernels per hill resulted in hills containing 1, 2, 3, 4, 5, and 6 plants with 3.3, 16.0, 29.0, 30.2, 18.4, and 3.1 percent of the population within the respective hill types.

The data in tables 15 and 16 show essentially the same facts for both the systematically and randomly variable distributions: That the plants grown in 1- and 2-plant hills had relatively fewer barren plants, and conversely more plants bore ears than in hills containing 4, 5, and 6 plants, while 3-plant hills were intermediate. In every instance the mean height of mature plants grown in 1-plant hills was greater than for plants grown in 2-, 3-, 4-, 5-, or 6-plant hills, and this difference tended to become larger as the number of plants per hill increased. The mean weight of shelled corn per ear-bearing plant was greatest for hills containing only one plant and as the number of plants per hill increased the shelled-corn weight per plant tended to decrease rapidly. When the average weight of shelled corn for all plants was considered, the differences were more striking since the decreases for large hills were augmented by a higher number of barren plants.

In general, these data from uneven stands show the plant size and productivity to be in inverse relation to hill size.

DISCUSSION AND CONCLUSIONS

The irregular stands of dent corn under investigation were fully as variable as might ever be expected to occur in the field under practical farm conditions as a result of mechanical imperfections of the planter or the use of imperfectly germinating seed. The data are based on (1) three systematically variable stands, (2) three randomly variable stands, and (3) essentially uniform stands grown for comparison. All of these approximated an average of three plants per hill. The systematic distributions consisted of alternating hills with (1) 2 and 4 plants, (2) 1, 2, 3, 4, and 5 plants, and (3) 1, 3, and 5 plants, respectively. The randomly variable distributions were obtained by planting seed corn germinating 75, 60, and 50 percent at adjusted rates to average three sound seeds per hill.

It is shown that through application of the law of chance a very close estimation of the resulting distribution of viable seeds may be had before planting, when the germination percentage of the seed to be planted and number of kernels to be dropped per hill are known.

During a 14-year period, the systematically variable stands averaged 50 bushels per acre as compared with 49.9 bushels for the uniform stand. The most variable stand yielded 0.7 bushel or 1 percent less than the uniform stand.

In the unusually dry year of 1931, seed germinating 100, 75, 60, and 50 percent, planted at the respective rates of 3, 4, 5, and 6 seeds per hill, gave corresponding yields of 24.96 ± 0.65, 25.50 ± 0.66, 25.34 ± 0.66, and 25.12 ± 0.65 bushels per acre. Differences between these are not statistically significant, and it is concluded that these stand irregularities have not affected yields.

The influence of these various stand irregularities upon the uniformity of plant development as measured by the standard deviation and coefficient of variation was determined in 1931 for the following characters: Plant height at several stages of development, ear height, stalk diameter, date of silking, and yield of grain per ear-bearing

plant. In nearly all cases the standard deviations and coefficients of variation were greater for the irregular stands, and frequently the increase was statistically significant though not large and of no great agronomic importance.

In the majority of cases the mean values for the various plant characters observed were slightly reduced by stand irregularity.

The effect of uneven stands upon the uniformity of plant development becomes more strikingly apparent if the plants grown within the various hill types are compared, since performance tended to vary inversely as the number of plants per hill. To illustrate: In the random distribution secured from corn germinating 50 percent, planted at 6 kernels per hill, the plants grown in 1-, 2-, 3-, 4-, 5-, and 6-plant hills yielded, respectively, approximately 107, 100, 73, 66, 63, and 48 g of moisture-free grain per ear-bearing plant. Similarly, the average mature height of plants grown in 6-plant hills was only 90 percent as great as for those grown in 1-plant hills, and the intervening hill types fell almost regularly between these extremes.

In general, it is evident that there may be considerable irregularity in the distribution of plants in a cornfield without the yield of grain per acre being materially affected. Such unevenness in stands, however, tends to increase the variability of plant development. Maximum uniformity among plants may be achieved through seed selection and planting practices that are conducive to uniformity of stand. Experience has indicated that stand irregularities materially greater than those herein considered, such as are sometimes caused by rodents, worms, birds, and soil washing, would undoubtedly increase the plant variability and lower the yield.

INFLUENCE OF PASTURE MANAGEMENT UPON THE GRAZING HABITS OF DAIRY CATTLE[1]

By R. E. HODGSON

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INTRODUCTION

Any improvement in pasture management that will cause lactating cows to obtain their sustenance with a minimum of grazing will conserve their energy for milk production. Energy expended in work incurred as a result of excess grazing is used at the expense of production, particularly in cases where herbage is sparse and supplemental barn feeding is not the general practice.

Few investigators have studied the physical activities of dairy cattle on pasture. Shepperd[2] made observations on three Hereford steers over a period of 24 hours, and reported that when on sweetclover pasture they averaged 8¼ hours' grazing, 7¼ hours' lying, 4 hours' sleeping, and 4½ hours' loafing (standing, not eating). Moseley, Stuart, and Graves[3] reported that cows receiving pasture alone grazed on an average 9.16 hours per day, while animals receiving alfalfa in addition to pasture grazed on an average 6.50 hours, or 29 percent less. Cows receiving a limited-grain ration (1 pound of grain for every 6 pounds of milk produced) and cows receiving a full-grain ration (1 pound of grain for every 3 pounds of milk produced) grazed 39 and 42 percent less, respectively, than those on pasture alone. These observations were made between 5 a.m. and 9 p.m. on 2 days in July and on 2 days in August. Fuller[4] concluded that cows kept in the barn during the winter stood approximately 50 percent of the time. As a result of studying the habits of 22 cows he found that approximately 3 hours a day was spent in eating.

The investigation described in this paper was undertaken to gain knowledge of the physical activities of lactating cows subjected to different types of grazing.

EXPERIMENTAL PROCEDURE

The data presented in this paper were obtained from an experiment at the Western Washington Experiment Station, conducted for the purpose of determining the efficiency of rotational grazing.[5]

Eight Holstein cows in about the same stage of lactation were divided into two lots as nearly alike as possible in respect to age, live weight, gestation, and production. Lot 1, consisting of four

[1] Received for publication Mar. 30, 1933; issued October 1933. Scientific paper no. 255, College of Agriculture and Experiment Station, State College of Washington.

[2] SHEPPERD, J. H. SWEET CLOVER EXPERIMENTS IN PASTURING. N.Dak. Agr. Expt. Sta. Bul. 211, 57 p., illus. 1927.

[3] MOSELEY, T. W., STUART, D., and GRAVES, R. R. DAIRY WORK AT THE HUNTLEY FIELD STATION, HUNTLEY, MONT., 1918–1927. U.S. Dept. Agr. Tech. Bul. 116, 48 p., illus. 1929.

[4] FULLER, J. M. SOME PHYSICAL AND PHYSIOLOGICAL ACTIVITIES OF DAIRY COWS UNDER CONDITIONS OF MODERN HERD MANAGEMENT. N.H. Agr. Expt. Sta. Tech. Bul. 35, 30 p., illus. 1928.

[5] "Rotational grazing" is defined as restricted grazing of animals rotating over units of the entire area.

animals, was subjected to continuous grazing [6] on a 2-acre pasture. Grazing was maintained at the rate of two cows per acre during a grazing season of 147 days. Lot 2 was subjected to rotational grazing on a similar 2-acre pasture divided into six ⅓-acre plots. On each plot, grazing was maintained at the rate of 12 cows per acre. The carrying capacity of the rotation pasture was at the rate of two cows per acre for the 147 days. The frequency of rotation was governed by the rapidity with which the plots were grazed to a uniform height of about 1 inch.

Except for the method of grazing, both lots were managed identically. Each animal received a supplemental low-protein grain ration, at the rate of 1 pound for every 7 pounds of milk produced.

Records were kept of the length of time spent by each cow in grazing and lying and the number of times that grazing and lying occurred. Information was obtained for 5 different weeks at approximately monthly intervals, beginning April 28, May 28, June 27, July 30, and September 8. During the weeks of observation an attendant was on watch each day from 6 a.m. until dark. The cows were removed from the pasture at 4 a.m., at which time there were seldom any cows grazing. They were returned to the pasture at 6 a.m. During the afternoon milking they were off the pasture from 2 p.m. until 5 p.m. No data were taken after dark. Except during the week beginning September 8, all cows had discontinued grazing and were apparently settled for the night before the attendant left the field.

PRESENTATION OF RESULTS

Table 1 shows the amount of time spent in grazing and lying and the number of times that grazing and lying occurred for the individual cows subjected to the two types of grazing. The means are calculated from five different weekly observation periods made at approximately monthly intervals.

For cows subjected to continuous grazing the average time expended daily in grazing and lying was 439.10 minutes and 154.02 minutes, respectively, as compared with 411.08 minutes and 192.40 minutes, respectively, for cows subjected to rotational grazing. The cows subjected to rotational grazing obtained their sustenance in an average of 28.02 minutes less than did those grazing promiscuously over the large pasture. They were reclining on average 38.38 minutes more than cows on the open pasture.

The average number of times that grazing and lying occurred was slightly greater for the cows on the rotational-grazing system. The time expended in loafing (standing not eating) was 68.30 minutes per day for the cows on the rotation system, as compared with 82.88 minutes for the other cows. The shorter time expended in grazing and loafing and the increase in time of resting indicates that the cows subjected to rotational grazing were able to obtain their sustenance with a smaller expenditure of energy than those whose grazing was unrestricted.

Figure 1 depicts the activities of the grazing animals as the season advanced. The average number of minutes the cows were under observation per day varied with the time at which darkness set in. As the grazing season advanced the average time expended in grazing

[6] "Continuous grazing" as used in this report refers to the unrestricted grazing of animals over the entire pasture area.

TABLE 1.—*Average amount of time cows on continuously grazed pasture and on rotationally grazed pasture spent per day in grazing and in lying, and the number of times grazing and lying occurred*

LOT 1, COWS ON CONTINUOUSLY GRAZED PASTURE

Cow no.	Number of days under observation	Observation time per day (minutes)			Time spent in grazing per day (minutes)			Number of times grazing occurred per day			Time spent in lying per day [a] (minutes)			Number of times lying occurred per day		
		Mean	Probable error	Standard deviation	Mean	Probable error	Standard deviation	Mean	Probable error	Standard deviation	Mean	Probable error	Standard deviation	Mean	Probable error	Standard deviation
122	35	676.00	±1.004	8.80	432.50	±1.684	14.82	8.66	±0.304	2.67	151.75	±1.076	8.44	2.39	±0.083	0.66
126	35	676.00	±1.004	8.80	427.50	±1.365	11.97	7.34	±.219	1.92	185.72	±1.924	10.15	2.93	±.195	1.53
134	35	676.00	±1.004	8.80	449.00	±1.460	12.81	8.20	±.299	2.62	148.22	±1.125	8.82	2.14	±.011	.88
139	35	676.00	±1.004	8.80	445.35	±1.695	14.87	7.29	±.219	1.92	130.35	±1.141	8.95	2.07	±.039	.304
Mean		676.00	±.542	8.80	439.10	±.852	13.84	7.87	±.145	2.35	154.02	±.634	9.94	2.38	±.071	1.12

LOT 2, COWS ON ROTATIONALLY GRAZED PASTURE

214	35	671.78	±0.945	8.29	444.65	±0.978	8.58	8.03	±0.486	2.52	147.52	±1.740	13.65	2.18	±0.036	0.28
125	35	671.78	±.945	8.29	406.08	±1.093	9.59	10.66	±.425	3.73	188.40	±1.373	10.77	3.29	±.080	1.25
142	35	671.78	±.945	8.29	407.50	±1.114	9.77	7.80	±.205	1.80	207.15	±1.399	10.97	3.54	±.129	2.03
146	35	671.78	±.945	8.29	390.35	±.931	8.17	9.46	±.310	2.72	226.78	±1.297	10.17	3.50	±.088	1.38
Mean		671.78	±.511	8.29	411.08	±.610	9.89	8.83	±.150	2.44	192.40	±.823	12.91	3.13	±.098	1.54

[a] Based on 28 days of observation.

by the cows on the rotationally grazed pasture decreased from 414 minutes per day during the week of April 28 to 385.8 minutes during the week of September 8. During the same period the average grazing time per day for the cows on the open pasture increased from

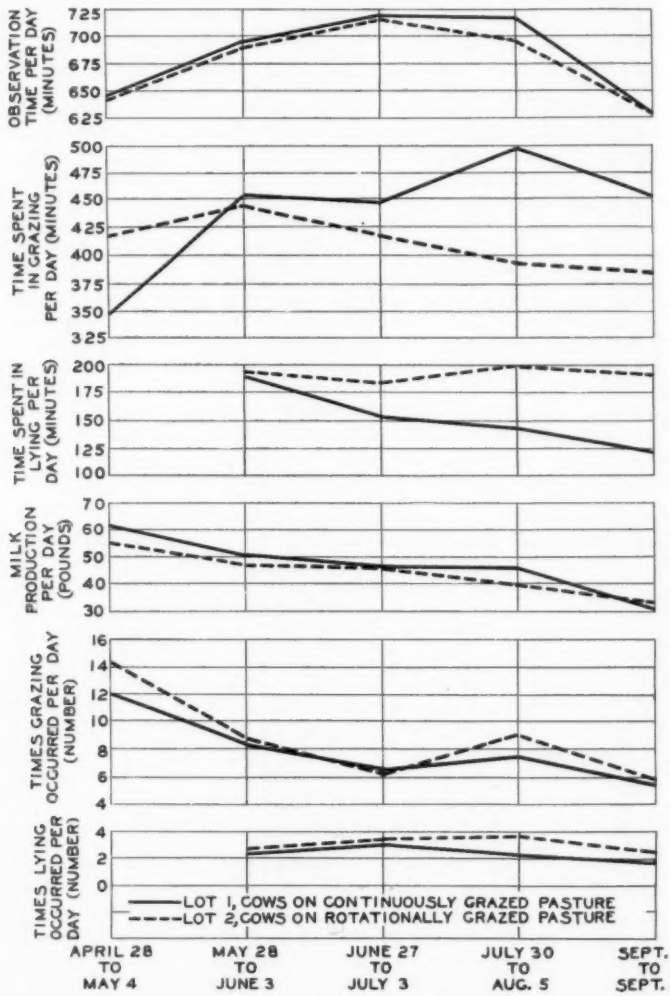

FIGURE 1.—Effect of advancement in season upon the grazing and lying activities of lactating cows.

339.28 minutes to 455.35 minutes. The greater amount of time spent in eating by the cows on rotationally grazed pasture during the first week of observation may have been the result of their grazing over two plots in succession, whereas the other group had access to an abundance of grass throughout the period.

From the week beginning May 28 to the week of September 8, the decrease in the average time spent in lying was 66.98 minutes for the cows on the continuously grazed pasture as compared with 8.05 minutes for the cows on the rotationally grazed pasture. The number of times that lying occurred also decreased more in the case of the cows grazing unrestrictedly. The trend of milk production remained practically the same for the two lots throughout the season.

As the season advanced the cows on the rotational-grazing system expended less energy in grazing. This did not cause a significant increase in their milk production, but it did result in a greater gain in live weight as compared with that of the cows grazing over the open pasture.

Intensive systems of pasture management require that rotation units be closely grazed in order that the herbage may be kept succulent and in a highly nutritious condition. It is difficult, however, to determine the optimum number of days that milking cows should be maintained on a rotation unit in order to accomplish uniform close grazing without decrease in live weight or milk production. Table 2 shows the effect of increasing the number of days that cows are maintained on a rotation plot on their grazing and lying activities. Observations were made while cows were maintained on individual plots for 1, 2, 3, and 4 days. The cows grazed on an average 1.8 percent more of the total time observed on the fourth day than on the first.

Table 3 shows the effect on live weight of increasing the number of days that cows are maintained on a rotation plot. Individual live weights of the four cows in lot 2 were recorded over a period of 45 consecutive days, 15 of which were taken at the end of the first day on a rotation plot, 15 at the end of the second day, and 13 at the end of the third day. There was a significant relation between loss in live weight and increasing days on a rotation plot. This is evidenced by the average live weight loss of 10.49 pounds the second day and 14.59 pounds the third day as compared with the first day.

Table 4 shows the effect on milk production of increasing the number of days that cows are maintained on a rotation plot. A comparison between milk production and the number of days that cows were maintained on a plot was made for the 1931 and 1932 seasons.

The evening milk production (the last milking of the experimental day) and the total day's production (including the evening milking plus the following morning milking) were used in making this comparison.

The data show that milk production decreased as the number of days that cows were maintained on a rotation unit increased. This relationship was more pronounced during the 1932 season when cows were maintained on individual plots for as many as 4 days. Reduction in the amount of milk produced as a result of increasing the number of days that the cows were on a rotation unit was evidenced in both the evening milking and the total day's production.

TABLE 2.—*Effect of increasing the number of days that cows are maintained on a rotation plot on their grazing and lying activities*

Items of comparison	First day on plot (minutes)			Second day on plot (minutes)			Variation from first day (percent)	Third day on plot (minutes)			Variation from first day (percent)	Fourth day on plot (minutes)			Variation from first day (percent)
	Mean	Probable error	Standard deviation	Mean	Probable error	Standard deviation		Mean	Probable error	Standard deviation		Mean	Probable error	Standard deviation	
Length of observation period	657.95	±1.635	10.18	675.00	±0.780	6.98	±2.59	687.50	±0.699	5.86	+4.49	672.50	±1.128	7.48	+2.21
Time spent in grazing per day [a]	385.80	±.948	9.32	402.50	±.864	7.73	±4.33	403.90	±.973	8.16	+4.69	406.25	±1.994	13.22	+5.30
Times grazing occurred per day—number	7.68	±.235	2.31	7.55	±.285	2.55	−1.69	10.81	±.508	4.26	+40.75	11.20	±.531	3.52	+45.83
Time spent in lying per day [b]	212.50	±1.373	12.28	175.15	±1.566	13.13	17.58	186.54	±1.473	10.70	−12.22	158.32	±3.201	16.44	−25.50
Times lying occurred per day—number	3.20	±.146	1.31	2.78	±.170	1.43	−13.12	2.88	±.150	1.09	−10.00	3.25	±.215	1.36	+1.56

[a] Data on grazing involved a total of 44 cow-days for the first day on a pasture plot, 40 for the second day; 32 for the third day, and 20 for the fourth day.
[b] Data on lying involved a total of 40 cow-days for the first day on a pasture plot, 32 for the second day, 24 for the third day, and 12 for the fourth day.

TABLE 3.—*Effect of increasing the number of days that cows are maintained on a rotation plot on their average live weight*

Cow no.	Weight (pounds) first day on plot [a]			Weight (pounds) second day on plot [a]			Decrease	Weight (pounds) third day on plot [b]			Decrease
	Mean	Probable error	Standard deviation	Mean	Probable error	Standard deviation		Mean	Probable error	Standard deviation	
214	1,305.00	±1.189	6.83	1,289.00	±1.227	7.33	16.00	1,286.54	±1.500	8.02	18.46
125	1,413.67	±1.062	6.10	1,406.33	±1.062	6.10	7.34	1,406.54	± .980	5.24	7.13
142	1,164.93	±.994	5.71	1,156.33	±1.170	6.72	8.60	1,150.38	±.660	3.53	14.55
146	1,221.67	±.869	4.99	1,211.67	±1.057	6.07	10.00	1,203.46	±1.109	5.93	18.21
Average	1,276.32	±1.028	5.91	1,265.83	±1.129	6.56	10.49	1,261.73	±1.062	5.68	14.59

[a] Data are based on 15 days. [b] Data are based on 13 days.

The effect of increasing the number of grazing days per plot upon the physical activities, live weight, and production of lactating cows illustrates the disadvantage of requiring heavy-milking cows to graze the area uniformly close before they are moved to the next unit of the rotation series. It also indicates the advisability of allowing the heavy-milking cows to graze the choicest portion of the grass, to be followed by dry stock and heifers that must obtain their sustenance by close grazing.

TABLE 4.—*Effect on milk production of increasing the number of days that cows are maintained on a rotation plot, 1931 and 1932*

Year and items of comparison	Number of cow-days	Milk yield (pounds) first day on plot			Number of cow-days	Milk yield (pounds) second day on plot			Variation from first day (percent)
		Mean	Probable error	Standard deviation		Mean	Probable error	Standard deviation	
1931									
Milk production:									
Evening milking—									
Apr. 30 to July 2	119	20.52	±0.298	4.82	106	19.57	±0.318	4.76	−4.63
July 3 to Sept. 9	91	14.08	±.279	3.95	96	14.02	±.286	4.15	−.43
Average	105	17.30	±.288	4.38	101	16.80	±.302	4.46	−2.89
Evening and morning milking [a]—									
Apr. 30 to July 2	119	41.16	±.567	9.17	106	38.36	±.610	9.31	−6.80
July 3 to Sept. 9	91	28.88	±.549	7.77	96	27.94	±.600	8.24	−3.25
Average	105	35.02	±.558	8.47	101	33.15	±.605	8.78	−5.34
1932									
Milk production:									
Evening milking—									
Apr. 28 to July 5	88	23.27	±.232	3.22	88	23.05	±.227	3.15	−.95
July 6 to Sept. 13	96	17.54	±.164	2.38	92	17.39	±.160	2.28	−.86
Average	92	20.40	±.189	2.80	90	20.22	±.194	2.72	−.88
Evening and morning milking [a]—									
Apr. 28 to July 5	88	51.10	±.535	7.17	88	48.52	±.522	7.00	−5.05
July 6 to Sept. 13	96	38.20	±.324	4.71	92	36.68	±.334	4.69	−3.98
Average	92	44.65	±.430	5.94	90	42.60	±.428	5.84	−4.59

[a] Includes the evening milk production on the day in question plus the following morning's production.

TABLE 4.—*Effect on milk production of increasing the number of days that cows are maintained on a rotation plot, 1931 and 1932*—Continued

Year and items of comparison	Number of cow-days	Milk yield (pounds) third day on plot: Mean	Probable error	Standard deviation	Variation from first day (percent)	Number of cow-days	Milk yield (pounds) fourth day on plot: Mean	Probable error	Standard deviation	Variation from first day (percent)
1931										
Milk production:										
Evening milking—										
Apr. 30 to July 2	48	20.04	±0.301	3.09	−2.34					
July 3 to Sept. 9	30	13.73	±.514	4.18	−2.49					
Average	39	16.88	±.408	3.64	−2.43					
Evening and morning milking [a]—										
Apr. 30 to July 2	48	40.21	±.818	8.41	−2.31					
July 3 to Sept. 9	30	28.86	±1.172	9.52	−.07					
Average	39	34.54	±.995	8.96	−1.37					
1932										
Milk production:										
Evening milking—										
Apr. 28 to July 5	68	21.85	±.262	3.21	−6.10	32	22.44	±0.371	3.11	−3.57
July 6 to Sept 13	80	16.52	±.159	2.11	−5.82	12	15.67	±.316	1.56	−10.66
Average	74	19.18	±.210	2.66	−5.98	22	19.06	±.344	2.34	−6.57
Evening and morning milking [a]—										
Apr. 28 to July 5	68	48.74	±.581	6.91	−4.62	32	48.50	±.887	6.67	−5.09
July 6 to Sept. 13	80	35.35	±.346	4.58	−7.46	12	34.34	±.724	3.76	−10.10
Average	74	42.04	±.464	5.74	−5.84	22	41.42	±.806	5.22	−7.23

[a] Includes the evening milk production on the day in question plus the following morning's production.

CONCLUSION

Data are presented which show the comparative grazing habits of cows subjected to two types of pasture management.

Cows subjected to rotational grazing, on an average grazed 28.02 minutes less and spent 38.38 minutes more in lying than similar cows on an open pasture where unrestricted grazing occurred. Cows on the rotational system expended an average of 411.08 minutes daily in grazing and 192.40 minutes in lying. The average number of times that grazing and lying occurred in this lot was 8.83 and 3.13, respectively, as compared with 7.87 and 2.38 for those on the continuously grazed pasture.

As the grazing season advanced the cows on the rotational system expended less time in grazing and more in lying, whereas those on the continuously grazed pasture spent more time in grazing and less in lying.

Increasing the number of days that cows were maintained on a rotation unit effected a marked increase in the number of times that grazing occurred but did not materially increase the average time expended in grazing. Cows that grazed on a plot for 4 days were lying on an average 25.50 percent less on the fourth day than on the first.

A decrease in live weight accompanied an increase in the number of days that the cows were maintained on a pasture unit. The average decrease at the end of the second and the third day was 10.49 pounds and 14.59 pounds, respectively.

There was a significant decrease in milk production, which corresponded with an increase in the number of days that the cows were maintained on a rotation unit.

TRUE AND APPARENT DIGESTIBILITY OF OAT HULLS AND ALFALFA MEAL BY SWINE, WITH SPECIAL REFERENCE TO THE ABILITY OF SWINE TO DIGEST CELLULOSE AND CRUDE FIBER.[1]

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INTRODUCTION

Although oat hulls are used to a considerable extent in swine feeding, as a constituent part either of the whole oat grain, or of oat feed, or of commercial mixed feeds, their digestibility by swine has never been determined, insofar as the writers are aware. They are not ordinarily accorded any high feeding value, mainly because of their high content of crude fiber. Also, no determinations of the digestibility of alfalfa hay by swine appear to have been reported in the literature.

The ability of swine to digest the crude fiber of feeds has been variously reported, the coefficients of digestion ranging from 10 to 90. This is probably due to differences in composition of the constituents making up the crude fiber of the various feeds, as well as to differences in the method of analysis for crude fiber. It is commonly supposed that lignocelluloses are not appreciably digested by swine, or by other animals with a digestive tract affording no opportunity for the stagnation and extensive fermentative decomposition of feed. It seems reasonable to expect, on the other hand, that the hemicelluloses, found to a variable extent in the crude fiber, will be broken down more readily by the intestinal bacteria than any other component of this fraction of carbohydrates. It would seem, however, that these more soluble constituents in crude fiber may be responsible for coefficients of digestion possessing a purely fictitious significance. If, during their passage through the digestive tract, they become more soluble in the acid and alkali used in the crude-fiber determination, because of the prolonged action of the slight acidity of the gastric juice, or possibly to a slight but incomplete disintegration by bacteria, they will be included in the nitrogen-free extract of the feces rather than in the crude-fiber fraction to which they originally belonged. This change in classification would occasion an increase in the estimated digestibility of crude fiber and a decrease in that of nitrogen-free extract, both effects of course having no biological significance. Possibly all digestion coefficients for the crude fiber of feeds are more or less high, because of this change in solubility of some of the compounds included therein during their passage through the intestinal tract of an animal.

REVIEW OF LITERATURE

The published evidence concerning the digestibility of the true celluloses of feeds by swine has been interpreted to mean that swine are not inferior to ruminants in this respect. The evidence, however,

[1] Received for publication May 8, 1933; issued October 1933.

is not impressive. In 1913 Fingerling and his associates (*2*) [2] reported the results of a series of digestion trials with 2 wethers and 2 pigs, an alkali-digested straw pulp, immature grass, and wheat chaff being used as sources of cellulose. The average digestibility of crude fiber in the straw pulp in the case of the pigs was almost 95 percent and was appreciably higher than the coefficients obtained in the trials with the wethers. The crude fiber of the other feeds was digested much better by the wethers than by the pigs. The authors explain the high digestibility of the crude fiber of straw pulp by pigs on the assumption that it was practically pure cellulose, while the crude fiber of the grass and the wheat chaff was lignified to a great extent.

Similarly Woodman and his associates studied the digestibility of sugar beets (*18*), and of sugar-beet pulp (*17*), using two pigs in each trial. Average digestion coefficients of 90 and 84, respectively, were obtained for the crude fiber. Commenting on these results, the authors (*17, p. 660*) say:

> The capacity of pigs to deal with fibre is usually regarded as very limited. The present results, however, show that this capacity is dependent on the nature of the fibre in the feedingstuff, and that when the fiber is non-lignified (that is, when it is composed mainly of cellulose) then such fibre may be digested to a very high degree.

The difficulty in interpreting both of these experiments in terms of cellulose is the uncertainty that the crude fiber either of the treated straw pulp or of the sugar-beet pulp is cellulose. In the first case, the alkali treatment may have initiated hydrolytic changes in the cellulose that would make it more amenable both to bacterial fermentation and to further hydrolytic cleavages in the intestinal tract of the pig, so that it would, in large part, pass from the crude-fiber constituents of the feed to the nitrogen-free extractives of the feces. In the second case, the crude fiber of the sugar-beet pulp may consist largely of the more difficultly soluble hemicelluloses rather than of cellulose, for all that is known to the contrary.

The majority of experiments concerned with the digestion of cellulose by other animals than the pig that possess a simple digestive tract indicate a very inconsiderable digestibility. Reference may be made in particular to the work of Scheunert and Lötsch (*15*) and of Thomas and his associates (*16*) on the dog, the work of Von Knierem (*9*) and Henning (*6*) on poultry, and of Fölling (*3*) on men.

EXPERIMENTAL PROCEDURE

The primary purpose of the experiment reported in this paper was the determination of the true digestibility of oat hulls by swine. This was accomplished in two series of digestion trials. In the first trials, the ration fed contained 30 percent of oat hulls and 70 percent of other constituents presumably completely digestible, i.e., 62 percent of cornstarch, 5 percent of sucrose, and 3 percent of a mineral mixture that contained the following ingredients: Steamed bone meal, 30 percent; ground limestone, 30 percent; NaCl, 30 percent; $MgCO_3$, 3 percent; K_2CO_3, 3 percent; K_2SO_4, 2 percent; $FeCl_2$, 1.5 percent; and KI, 0.5 percent.

In the second series of trials the ration contained, in place of the oat hulls, a mixture of cellulose and starch, the former equalling the

[2] Reference is made by number (italic) to Literature Cited, p. 434.

crude-fiber content of 30 percent of oat hulls. This ration contained 83.95 percent of starch, 8.05 percent of dried paper pulp (cellomass) or cellophane, 5 percent of sucrose, and 3 percent of the same mineral mixture. The purpose of the second ration, fed in the same amounts as the first, was to measure the metabolic products in the feces, which are presumably (*11, p. 21*) related to the intake of dry matter and to its content of crude fiber.

The experimental animals were 4 barrows, whose weights averaged 168, 175, 178, and 185 pounds respectively, for the entire period of the digestion trials. The 2 lightest pigs belonged to the Duroc-Jersey breed, and the 2 heaviest belonged to the Poland China breed. The pigs were practically constant in weight throughout the experiment.

The collection periods were 10 days long and were preceded by preliminary periods of at least 4 days, during which the same amount and kind of feed was consumed. All animals in all the periods consumed 1,200 g daily of the experimental rations in two equal portions given in the morning and late afternoon. There was no refused feed at any time.

A single digestion period on alfalfa meal was completed on 1 pig only, at the end of the 2 experimental periods described above, 3 of the pigs refusing to consume the alfalfa ration in sufficient amounts. The alfalfa-meal ration was patterned after the oat-hull ration, the substitution of one feed for the other being made in such a way that the crude-fiber content of the rations would be approximately the same.

The feed, feces, and urine were analyzed by the methods of the Association of Official Agricultural Chemists (*1*), except for crude fiber, which was determined by a modification of a method devised by Forbes and his associates (*4*). Since the digestibility of crude fiber is of primary interest to this study, the method used for its determination is given in detail.

A sample of feed or feces of 1½ to 3 g, depending on the crude-fiber content, was weighed out, 200 cc of 1.25 percent boiling sulphuric acid was added, and the mixture boiled for 30 minutes under a reflux condenser. Then 200 cc of 3.52 percent boiling sodium hydroxide solution was added and the mixture again boiled for 30 minutes. It was filtered on a Buchner funnel through fine linen (tracing cloth), moderate suction being used if necessary, and it was washed with hot water until the washings were free of chlorides. The residue on the linen was transferred to a beaker with water and filtered through a Gooch crucible provided with a pad of asbestos. The crucible and contents were dried by heating in an oven overnight at a temperature of 105° C., weighed, ignited, and weighed again, the difference in weights representing the crude fiber.

In the writers' experience there was no advantage in extracting the dried crude fiber with fat solvents. Apparently, in all ordinary samples at least, the fat in the sample is completely saponified during the 30-minute treatment with acid and alkali.

EXPERIMENTAL RESULTS WITH OAT HULLS

The oat hulls used in this experiment were prepared from whole oats by the Division of Swine Husbandry through the courtesy of Dr. W. E. Carroll. Care was taken to exclude practically all unhulled

kernels. The chemical analysis of the oat hulls gave the following results: Moisture, 8.05 percent; crude protein, 4.52 percent; ether extract, 3.28 percent; ash, 7.20 percent; crude fiber, 26.82 percent; and nitrogen-free extract, 50.13 percent. The gross-energy content, determined with the bomb calorimeter, was 3.98 calories per gram.

The coefficients of apparent digestibility of the oat-hull ration, as determined on the ordinary assumption that all the constituents of the feces are indigestible residues of the feed, are given in table 1.

TABLE 1.—*Coefficients of apparent digestibility of the oat-hull ration when eaten by swine*

Pig no.	Coefficients of apparent digestibility					
	Dry matter	Crude protein	Nitrogen-free extract	Crude fiber	Ether extract	Gross energy
2-PC	74.4	49.8	85.0	10.3	64.7	74.2
3-DJ	74.3	48.0	85.1	8.8	60.0	73.8
4-PC	75.6	47.5	85.9	14.7	66.6	75.0
5-DJ	76.0	46.4	86.6	15.6	48.3	75.6

When the nitrogen-free rations were made up for the second series of digestion trials, it was assumed that both the paper pulp (cellomass) and the cellophane, serving as crude fiber, consisted entirely of cellulose. On analysis of the rations, however, it was found that while the cellomass ration contained 7.47 percent of crude fiber, the cellophane ration contained only 4.12 percent. The cellophane itself was found to contain only 55.20 percent of crude fiber. The remainder of the cellophane, as the writers have since found, consists mainly of glycerol.

The fecal constituents excreted while the pigs were on the low-nitrogen rations (which contained about 0.05 percent of nitrogen) were assumed to consist, except for the crude fiber, entirely of the so-called metabolic products originating from the body rather than from the food. The metabolic dry substance (excluding the crude fiber), the metabolic nitrogen, and the metabolic ether extract excreted per kilogram of dry matter consumed, are given in table 2 for each pig.

TABLE 2.—*Metabolic products excreted in the feces of swine per kilogram of dry matter consumed*

Pig no.	Dry matter	Nitrogen	Ether extract	Pig no.	Dry matter	Nitrogen	Ether extract
	Grams	*Grams*	*Grams*		*Grams*	*Grams*	*Grams*
2-PC	32.9	0.81	1.00	5-DJ	32.2	0.67	1.03
3-DJ	32.2	.75	1.67				
4-PC	35.3	.80	2.26	Average	33.1	.76	1.49

The amounts of crude fiber excreted when the pigs were on these rations were very nearly the same as the amounts of crude fiber ingested. Thus, for the two pigs on the cellomass ration, nos. 2-PC and 3-DJ, 89.6 g of crude fiber were consumed daily, and 81.4 and

86.6 g, respectively, were excreted in the feces. For the two pigs on the cellophane ration, nos. 4-PC and 5-DJ, 49.4 g of crude fiber were consumed daily and 54.4 and 48.6 g, respectively, were determined in the feces. The average coefficient of digestibility was only 3.5 percent.

The computation of the true digestibility of the oat hulls is given in table 3, which contains the pertinent data for pig 2-PC.

TABLE 3.—*Computation of the true digestibility of oat hulls for pig 2-PC*

[All weights are in grams per day and the energy is in calories per day]

Items	Crude protein	Nitrogen-free extract	Crude fiber	Ether extract	Gross energy
Nutrients in 360 g of oat hulls	16.27	180.5	96.5	11.81	1,431
Nutrients in feces	10.25	132.6	98.0	3.81	1,137
Nutrients in metabolic products	5.51	17.0	----	1.09	115
Undigested nutrients of oat hulls	4.74	115.6	98.0	2.72	1,022
Digested nutrients of oat hulls	11.53	64.9	0	9.09	409
Coefficients of true digestibility	70.9	36.0	0	77.0	28.6

In the first line of figures are given the weights of nutrients contained in 360 g of oat hulls, representing 30 percent of the 1,200 g of ration consumed. In the second line are the weights of constituents in the feces excreted per day. On the assumption that all other components of the ration but the oat hulls are completely digestible, these fecal constituents include (1) the undigested material from 360 g of oat hulls, and (2) the metabolic products produced by 1,200 g of the ration. In the third line of figures are the amounts of fecal constituents (after making certain corrections) passed daily while the pig was receiving 1,200 g of the nitrogen-free diet. The crude fiber excreted when the pigs were on this diet is not included, since it was assumed to represent undigested crude fiber and in fact approximated closely the amounts of crude fiber consumed. Small adjustments (the correction above referred to) were made for all constituents to allow for small differences in the dry-matter content of 1,200 g of the two rations. These values (third line) are taken to measure the excretions of metabolic constituents due to the consumption of 1,200 g of the oat-hull ration. Hence, the differences between the values in the second line and those in the third are estimates of the indigestible constituents from the 360 g of oat hulls consumed. These differences make up the fourth line of figures in table 3. Deducting the values in the fourth line (undigested nutrients of oat hulls) from the corresponding values in the first line (nutrients contained in oat hulls) gives the nutrients digested from the oat hulls (fifth line), and these, expressed as a percentage of the amounts of nutrients contained in the oat hulls, are the coefficients of true digestibility, given in the last line of the table.

The coefficients of true digestibility of oat hulls for the four pigs, computed as shown in table 3, are included in table 4. These coefficients represent the nutrients actually absorbed from the digestion of oat hulls. An average of 66 percent of the crude protein and only 30 percent of the gross energy is utilized in digestion. For 100 pounds of oat hulls there appear to be only some 29 pounds of total digestible nutrients and 2.99 pounds of digestible protein.

TABLE 4.—*Coefficients of true digestibility of oat hulls when eaten by swine*

Pig no.	Coefficients of true digestibility					Total digestible nutrients per 100 pounds of oat hulls
	Crude protein	Nitrogen-free extract	Crude fiber	Ether extract	Gross energy	
						Pounds
2-PC	70.9	36.0	0	77.0	28.6	26.9
3-DJ	66.2	36.0	0	78.7	27.6	26.9
4-PC	67.2	41.9	3.5	89.9	32.6	31.6
5-DJ	60.4	44.4	4.5	62.2	32.7	30.8
Average	66.2	39.6	2.0	76.9	30.4	29.0

For all practical purposes even these values are too high, since it is customary, and logical, to charge against a feed the metabolic fecal material lost during its digestion. This charge is included in the ordinary coefficients of (apparent) digestibility. In the present experiments, such coefficients may be obtained by adding to the estimated undigested residue of each nutrient 30 percent of the estimated metabolic excretion of the same nutrient (since the ration contained 30 percent of oat hulls), subtracting the sum from the amount of the nutrient contained in 360 g of oat hulls, dividing the difference by the latter amount, and multiplying by 100.

An illustration of the calculation may be given again from the data for pig 2-PC contained in table 3. The calculation of the coefficient of apparent digestibility for protein would be $\frac{16.27-(4.74+1.65)}{16.27}\times 100=60.7$, 1.65 being 5.51×0.30. A summary of the coefficients of apparent digestibility of oat hulls for the four pigs is given in table 5.

TABLE 5.—*Coefficients of apparent digestibility of oat hulls when eaten by swine*

Pig no.	Coefficients of apparent digestibility					Total digestible nutrients in 100 pounds [a]
	Crude protein	Nitrogen-free extract	Crude fiber	Ether extract	Gross energy	
						Pounds
2-PC	60.7	33.1	0	74.2	26.2	24.8
3-DJ	56.8	33.3	0	74.2	25.1	24.7
4-PC	57.3	38.6	3.5	83.7	29.8	29.0
5-DJ	52.1	41.4	4.5	59.4	30.3	28.7
Average	56.7	36.6	2.0	72.9	27.8	26.8

[a] Computed on the basis of the composition of oat hulls used in this investigation.

By applying these digestion coefficients to the chemical composition of the oat hulls, it appears that each 100 pounds contain 2.56 pounds of digestible crude protein, 18.35 pounds of digestible nitrogen-free extract, 0.54 pounds of digestible crude fiber, 2.39 pounds of digestible ether extract, and 26.83 pounds of total digestible nutrients.

The digestibility of oat hulls by swine may be compared with its digestibility by other classes of livestock insofar as such determinations have been made. A compilation of such determinations is given in table 6.

TABLE 6.—*Comparison of the digestibility of oat hulls by different species of farm animals*

Kind of animal	Animals in test	Digestion coefficients				Total digestible nutrients in 100 [a] pounds	Literature reference
		Crude protein	Nitrogen-free extract	Crude fiber	Ether extract		
	Number					*Pounds*	
Swine	4	57	37	2	73	27	
Sheep	2	12	33	50	14	32	(*10*)
Do [b]		39	59	56	77	52	(*5*)
Do	1	41	33	44	70	35	(*8*)
Horse	2	100	11	22	82	22	(*10*)

[a] Based on analysis of oat hulls given in this paper.
[b] Oat-hull clippings.

The great discrepancy among the results for sheep precludes the possibility of any comparison of this species with swine with respect to their ability to digest oat hulls. It is apparent, however, that the pig is greatly inferior to both the sheep and the horse in its ability to digest the crude fiber of oat hulls.

It will be noted, both in the oat-hulls experiments and the alfalfa-meal experiment described later, that the crude fiber of the corresponding rations was apparently digested somewhat better than that of the oat hulls or the alfalfa meal themselves, although these feeds presumably were the sole source of crude fiber in their respective rations. This was due to the fact that the rations in each case contained slightly more crude fiber than would be predicted from their content of oat hulls or alfalfa meal and the composition of these feeds. All the crude-fiber determinations, performed in triplicate, gave results agreeing satisfactorily. The discrepancy between the analysis of the feeds and of the rations may have been the result either of an imperfect mixing of the rations, so that somewhat nonrepresentative samples were obtained for chemical analysis, or of an interference of the starch and sugar with the crude-fiber determination. In either case, more confidence may be felt in the crude-fiber determinations of the feeds than of the rations. It is assumed that the constituents of the rations were properly weighed out.

The crude fiber of oat hulls is known to contain some pentosans (*14*) and considerable lignin (*13*) equivalent to 5.6 to 6.9 percent of the dried hulls.

During these digestion trials the urine as well as the feces was collected and was analyzed for nitrogen. Its gross energy content was also determined, a Parr oxygen bomb calorimeter being used for the purpose. The results of these determinations are given in table 7, together with the intake of nitrogen and energy and the nitrogen balances.

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TABLE 7.—*Daily metabolism of nitrogen and energy on the oat-hull ration and the low-nitrogen rations when eaten by swine*

Pig no.	Metabolism of—	Oat-hull ration				Low-nitrogen rations			
		Intake	Feces	Urine	Balance	Intake	Feces	Urine	Balance
2-PC	Nitrogen.....grams..	3.26	1.64	2.89	−1.27	0.58	0.87	2.45	−2.74
	Energy.....calories..	4,408	1,137	58	.....	4,336	439	40	.....
3-DJ	Nitrogen.....grams..	3.26	1.70	1.21	+.35	.58	.81	1.84	−2.07
	Energy.....calories..	4,408	1,156	24	.....	4,336	466	34	.....
4-PC	Nitrogen.....grams..	3.26	1.71	2.59	−1.04	.66	.86	2.71	−2.91
	Energy.....calories..	4,408	1,100	56	.....	4,252	355	47	.....
5-DJ	Nitrogen.....grams..	3.26	1.84	2.75	−1.33	.66	.72	2.66	−2.72
	Energy.....calories..	4,408	1,075	48	.....	4,252	307	50	.....

The fact that the urinary nitrogen of a pig on the oat-hull ration did not show a consistent increase over that for the same pig while on the low-nitrogen ration, indicates a very nearly complete utilization of the absorbed nitrogen of the oat hulls in replacing the endogenous losses of nitrogen. The intake of oat-hull nitrogen was insufficient, however, to establish equilibrium. The urinary-nitrogen excretions per day on the low-nitrogen rations, expressed in milligrams per kilogram of body weight, were 30.2, 23.0, 32.2, and 34.5, respectively. These are very close to the lowest observed endogenous nitrogen output of the pig (*12, p. 9*).

The gross energy of the urine was also about the same on the two rations. By assigning a proportionate share of this energy to the oat hulls, it may be estimated that the metabolizable energy averages 1,166 calories per kilogram of dry matter.

Oat feed, consisting largely of oat hulls, has been compared in feeding value with wheat bran in the feeding of dairy cows. For dairy cows oat feed is said to be worth 70 percent as much as wheat bran, in amounts not to exceed one fourth of the grain ration (*1a, p. 15*). For swine, oat hulls are worth much less than half as much as wheat bran. On the basis of average analyses for the two feeds and reported digestion coefficients for wheat bran (*7*) and those obtained here for oat hulls, wheat bran contains 58.3 pounds of total digestible nutrients and 12.0 pounds of digestible crude protein per 100 pounds, while oat hulls contain 24.8 pounds of total digestible nutrients and 2.3 pounds of digestible crude protein per 100 pounds.

EXPERIMENTAL RESULTS WITH ALFALFA MEAL

At the conclusion of the metabolism experiment involving the use of the low-nitrogen rations, an attempt was made to secure information concerning the digestibility of alfalfa hay. A ration similar to the oat-hull ration, but containing alfalfa hay in an amount to furnish the same percentage of crude fiber, was given the four pigs, the transition from the low-nitrogen rations being made gradually. However, only one of the pigs, no. 3-DJ, could be induced to consume 1,200 g of this ration daily, the amount in which the other rations were fed. Hence, the experiment on alfalfa hay was confined to this pig.

The alfalfa meal used in this test contained 10.50 percent of water, 13.44 percent of crude protein, 2.16 percent of ether-soluble material, 7.61 percent of ash, 29.26 percent of crude fiber, and 37.03 percent of

nitrogen-free extract. Its gross energy content was equivalent to 3.95 calories per gram. The ration in which this feed was fed contained 27.51 percent of alfalfa meal, 3.00 percent of the same mineral mixture used in the other rations, 5.00 percent of sucrose, and 64.49 percent of cornstarch. The same plan of feeding and length of feeding period were used as in the two preceding experimental periods.

The coefficients of apparent digestibility of the alfalfa ration for the one pig were: 79.7 for dry matter, 37.5 for crude protein, 94.0 for nitrogen-free extract, 10.8 for crude fiber, and 78.5 for gross energy. The ether extract contained in the feces (10.53 daily) exceeded that contained in the feed (7.80 daily). By calculating the digestibility of the alfalfa meal by the same methods that were used for oat hulls, the following coefficients of true digestibility were obtained: 45.9 for crude protein, 71.1 for nitrogen-free extract, 1.9 for crude fiber, and 38.6 for gross energy. Again, the digestibility of the ether extract could not be computed. According to these results, applied to the composition of the alfalfa meal used, there were 33.0 pounds of total digestible nutrients per 100 pounds of alfalfa meal.

The apparent digestibility of the alfalfa meal in the above ration is represented by the following coefficients: 42.7 for protein, 67.4 for nitrogen-free extract, 1.8 for crude fiber, and 36.1 for energy. The total digestible nutrients amounted to 31.3 pounds per 100 pounds of alfalfa meal.

The metabolism of nitrogen and energy of pig 3-DJ on the alfalfa ration and the nitrogen-free ration is summarized in table 8.

TABLE 8.—*Daily metabolism of nitrogen and of energy for pig 3-DJ on the alfalfa-meal ration and the low-nitrogen ration*

Metabolism of—	Alfalfa ration				Low-nitrogen ration			
	Intake	Feces	Urine	Balance	Intake	Feces	Urine	Balance
Nitrogen.....grams	7.45	4.66	2.48	+.31	0.58	0.81	1.84	−2.07
Energy.....calories	4,273	920	87	.....	4,336	466	34	.....

The protein content of the alfalfa hay was sufficient in quantity and quality to establish a slight positive nitrogen balance. The nitrogen of the urine was not greatly increased over that in the period in which the low-nitrogen ration was fed, indicating a very good utilization (82 percent) of the absorbed nitrogen. From the energy metabolism data, the metabolizable energy of the alfalfa meal may be computed at 1,624 calories per kilogram of dry matter.

SUMMARY AND CONCLUSIONS

The digestion and metabolism of four pigs were studied while they were consuming (1) a ration containing 30 percent of oat hulls, with starch, sugar, and salts equal to 70 percent, and (2) a ration planned to contain paper pulp or cellophane to furnish as much crude fiber as the oat hulls in the former ration, together with starch, sugar, and salts. In the case of one pig, a third ration containing about 30 percent of alfalfa meal, and the rest of starch, sugar, and salts was studied.

The total digestible nutrients of the oat hulls as ordinarily computed amounted to 26.8 pounds per 100 pounds and those of the alfalfa meal to 31.3 pounds per 100 pounds. The metabolizable energy contents were 1,166 and 1,624 calories per kilogram of dry matter, respectively.

At the low levels of protein feeding prevailing, the absorbed protein of both feeds was almost completely utilized in metabolism.

The crude fiber of the oat hulls was digested to an average extent of 2 percent and that of the alfalfa meal to practically the same extent. The presumably pure cellulose of the paper, as well as of the cellophane, was digested to the extent of only 3.5 percent, on an average, the range for individual pigs being from 0 to 9.2 percent.

It appears from these studies that the crude fiber of oat hulls and of alfalfa meal, as well as pure cellulose, pass through the digestive tract of the pig almost, if not entirely, untouched by bacteria or other agencies of digestion. A possible explanation of the results of other experiments, apparently indicating a considerable digestibility of crude fiber by swine, is offered.

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AN APPARATUS FOR THE READY DETERMINATION OF AREAS OF COMPOUND LEAVES[1]

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INTRODUCTION

A study made in 1932, involving extensive measurements of leaf areas in the black raspberry (*Rubus occidentalis* L.), required the development of a special technic. The blueprint and planimeter method had proved entirely too slow, and the matching method was not practicable because of the variability in shape of the leaflets. A method of direct translation, by reference to a table, of combined width plus length of leaflet into area was tested. This was based on a correlation ratio[2] of 0.972 between width plus length and area, found in 1,987 leaflets of fruiting canes, and a ratio of 0.994 found in 532 leaflets of current-season shoots. In practice this method required too much time. The leaf-punch method, though employed with great care and during most of the season in which the studies were made, gave values varying with different lots from 9 percent below to 19 percent above planimeter determinations; it was therefore not sufficiently accurate.

A correlation of 0.811 ± 0.095 was found between areas of 589 mid or end leaflets and the respective areas of compound or palmately trifoliate leaves, in fruiting canes. This indicated a close relationship between width plus length of end leaflets and area of compound leaves. Such corrected correlation ratios for 569 sample fruiting-cane leaves was 0.959 and for 407 leaves of current-season shoots it was also 0.959. These relationships indicated that a high degree of accuracy in area measurements of compound leaves would result from translating width plus length measurements of end leaflets into terms of leaf areas. To avoid tabular translation and to facilitate rapid and accurate measuring, the device here described was developed.

THE APPARATUS AND ITS UTILIZATION

The apparatus (fig. 1) consists essentially of two 50-cc hypodermic syringes (C) mounted at right angles to each other on the underside of a small table (D), the two syringes discharging into a common thick-walled rubber tube (E) (in later work a glass tube was used at E) connected with a vertical glass tube (A or B), which is mounted on a board calibrated in terms of areas of compound leaves. Attached to the pistons of the syringes are right-angle wires (F) which move across the field on the top of the small table (D) and on which the end leaflet is placed for measurement. When the pistons are so adjusted that the legs of the wires coincide with one side and the tip of an end leaflet, located at G, the area of the respective compound leaf is indicated on the scale at the top of the water column.

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[2] The curves for all correlation ratios in this paper are of the parabolic type, the relationships for the larger leaflets and leaves taking the form of a straight line.

The calibrated scale is such that when the pistons expel all the water from the syringes the top of the water column is at the top of the glass tube. This permits ready checking of the volume of water in the apparatus at frequent intervals.

The scale may be calibrated as follows: (1) Calculate the curve of best fit for width plus length of end leaflets and compound-leaf areas;

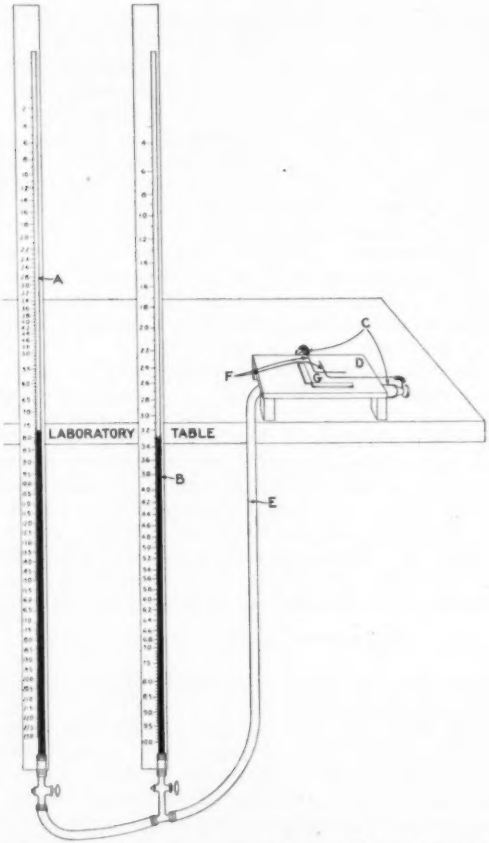

FIGURE 1.—Apparatus used in determining the areas of compound leaves of the black raspberry. The glass tube and calibration at A are designed to indicate the area of compound leaves of current-season shoots; those at B are designed to indicate the areas of the compound leaves of fruiting canes. See text for further description.

(2) alternately withdraw the plungers of the syringes one tenth inch at a time and record on the scale the corresponding compound-leaf areas indicated from the calculated curve; (3) interpolate; (4) the scale may then be further adjusted or corrected by using the apparatus to measure classes of end leaflets from compound leaves whose mean areas have previously been determined by the planimeter.

Inasmuch as the same relationships between width plus length of end leaflets and compound-leaf areas do not exist in leaves of fruiting canes and current-season shoots of the black raspberry, a scale for each must be calibrated. Furthermore, maximum individual leaf areas of current-season shoots are more than twice as great as those from fruiting canes.

Though the standard deviation between planimeter measurements and those obtained by the use of the apparatus amounted to 11 percent of the mean compound-leaf area for leaves of fruiting canes and 12.9 percent for leaves of current-season shoots, the total error [3] for 898 fruiting-cane leaves (a random sample from leaves collected between June 20 and July 29) was +3.7 square inches, or 0.15 percent, and the error for 373 leaves of current-season shoots was −11.2 square inches, or 0.55 percent. In measuring leaves from a single cane or shoot the error involved would probably be greater because of the smaller number involved. However, the method appears to approximate closely the accuracy of the planimeter, is much more rapid, and makes possible the handling of a much larger number of leaves, thus permitting more representative sampling.

[3] Total error refers to the total difference in areas as indicated by the planimeter and apparatus methods. For leaves of fruiting canes the indicated areas were 2,526. and 2,529.7 square inches, respectively, and for leaves of current-season shoots, 2,034.3 and 2,023.1 square inches, respectively.

THE RECOVERY OF SALMONELLA PULLORUM FROM THE FECES OF ADULT CARRIERS OF PULLORUM DISEASE[1]

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INTRODUCTION

The results of recent studies of pullorum disease of fowl indicate that adult bird carriers of this disease when confined with adult negative birds are a menace to the well-being of the negative birds. A slow progressive transmission of the infection in such cases has been demonstrated (*1, 2, 4, 5, 6, 9*).[2] Rettger and Plastridge (*8*), *p. 178*, after reviewing the work on this subject, state: * * * "It is safe to assume that *Bacterium pullorum* is readily transmitted from one adult bird to another through infected droppings." This paper offers additional proof that the causative organism of the disease can be recovered from the droppings of the adult carrier bird, and that such droppings may be a source of infection for adult negatives.

The importance of this problem is emphasized by the fact that in a number of States only a single annual test is applied to breeding flocks, and the detection and removal of reactors in this test are accepted as showing that a clean flock remains. The fallacy of this procedure was noted by Dearstyne, Greaves, and Gauger (*4*) in a report of the results of tests made by the North Carolina State Department of Agriculture in 1930. An initial test on 37,893 birds showed 3,194, or 8.43 percent, to be positive. Subsequent tests of those showing a negative reaction in the first test revealed 1,859 more reactors, bringing the total to 5,053, or 13.33 percent of the total number studied. These figures indicate that 36.8 percent of the total reactors remained in the flocks undetected by the first test. Obviously these birds were subjecting the adult negative birds to the chance of contracting the disease through contamination resulting from cohabitation. The results of experimental work with three flocks of reactors owned by the North Carolina Agricultural Experiment Station, showed that of 27 reactors, 12, or 44 percent, were intermittent to the test; of 26 reactors, 18, or 69.2 percent, were intermittent; and of 26 reactors, 19, or 73.1 percent, were intermittent.

The purpose of the investigation here described was to determine whether the carrier birds void *Salmonella pullorum* in their droppings, and if so, whether the recovered organisms are sufficiently virulent to produce the disease in negative adults and chicks when administered per os.

METHOD OF STUDY

The subjects used in this work were proved adult carriers that had been subjected to a semimonthly serological test for 2 years or more. The birds were brought from range and placed in individual

[1] Received for publication Jan. 3, 1933; issued October, 1933.
[2] Reference is made by number (italic) to Literature Cited, p. 445.

coops with wire bottoms, under which layers of clean paper were placed to catch the droppings.

During a period of 2 months 281 specimens of feces from 16 carrier birds were collected. These were placed in 4-ounce bottles containing 35 to 40 cc of meat-extract broth having a reaction of pH 6.6, and were incubated for 24 hours at 37° C. Approximately 1 cc of this broth was then inserted into sterile Petri dishes, and this was flooded with 2 percent base agar. The broth and agar were enriched with brilliant green in the proportion of 1:150,000. When the agar had solidified, the plates were inverted and incubated for 24 hours at 37° C. Various organisms, as judged by distinct colonies, were inserted into lactose fermentation tubes to which Andrade's indicator had been added. The results of these tests were negative for *Salmonella pullorum*.

Some difficulty in securing pure cultures was experienced in the preliminary tests, but this was overcome by improvement in technic. Two percent peptone water, as advocated by Rakieten and Rettger (7), was prepared. The hydrogen-ion concentration of this medium was so adjusted that it varied from pH 6.6 to 6.8 and was buffered with 1 percent of a 10-percent solution of potassium phosphate (KH_2PO_4 and K_2HPO_4) having a pH range of 6.6 to 6.8. Brilliant green was added to this medium in the proportions of 1:200,000, this dilution having been shown to inhibit the growth of colon organisms in cleared broth without causing serious suppression of the growth of *Salmonella pullorum* and *S. gallinarum*. Under aseptic conditions 5-cc amounts of this medium were placed in small test tubes. In these tubes fecal specimens one half to one fourth the size of a pea were planted. The inoculated broth was shaken thoroughly, placed in the incubator, and kept there for 6 to 8 hours at 37° C. After incubation, one half to 3 cc of the top fluid was poured into bottles containing 35 to 40 cc of cleared meat-extract broth to which brilliant green had been added, the amount used in each case depending on the turbidity present. The cultures were then incubated for 18 to 24 hours at 37° and streak tests made from them on brilliant-green agar plates. The streaked plates were inverted and incubated for 24 hours at 37°, and colonies from these cultures were inoculated into lactose fermentation tubes or into indicator plates prepared by the addition of 1 percent lactose and 1 percent Andrade's indicator to base agar. Three fecal samples were taken daily from the birds kept for study. Control cultures, seeded with *S. pullorum* and a fecal specimen from one of the carrier birds were used each day. In all, 236 fecal samples from 21 birds were examined.

RECOVERY OF THE ORGANISM

In this study *Salmonella pullorum* was recovered from the feces of two birds, designated by leg bands 1631 and 1862. *S. pullorum* was recovered from only 1 of 3 fecal samples taken on the same day from bird 1631; it was not recovered from 3 fecal samples taken on each of the sixth, seventh, tenth, and twelfth days subsequent to, nor on the day prior to, the recovery noted. *S. pullorum* was obtained from all of 3 fecal specimens taken from bird 1862 on the first day, but was not recovered from 3 fecal specimens taken on the first, fourth, and sixth days subsequent to the recovery of the organism.

The organisms were determined as *Salmonella pullorum* by the morphological, cultural, and biochemical properties of the strain as noted in the Report of the Conference of Official Research Workers in Animal Diseases (*3*).

The two birds from which the recoveries were made were Barred Plymouth Rocks and had the following histories:

Bird 1862 was hatched April 28, 1928. Its sire and dam were both reactors to tests for pullorum disease. The first positive test on this bird was secured 63 days after it was hatched. The bird reacted strongly to the test in all except 1 of 76 tests made at semimonthly intervals, the single negative test being secured when the bird was 13 weeks old. The serum titer ran 1:1,000 or higher in 26 of the 76 tests. There was a marked increase in positive trend of the serum titer as the bird matured. This bird was an intensive producer, having laid 519 eggs prior to the time of fecal examination. One hundred of these were positive for *Salmonella pullorum*. The 4 eggs laid by this bird immediately before the isolation of *S. pullorum* from the feces were positive for the organism.

Bird 1631 was hatched April 29, 1929. Its sire and dam were both reactors to tests for pullorum disease. The first positive test on this bird was secured 73 days after it was hatched. The bird was a reactor of the intermittent type, 36 of 64 agglutination tests made at semimonthly intervals being positive. The serum in all tests showed a titer that did not exceed a dilution of 1:50. Of 306 eggs laid and tested bacteriologically prior to the recovery of *Salmonella pullorum* from the feces, only 7 were positive for the organism. The serum of the bird was positive for *S. pullorum* agglutinins in a dilution of 1:25 in 4 tests made at 2-week intervals prior to the recovery of the organism from her feces.

INOCULATION TESTS

The three recoveries from bird 1862 were administered to three Single-Comb Rhode Island Red pullets that had proved negative for pullorum disease in three successive tests at 30-day intervals. The birds were inoculated per os with a 24-hour bacillary saline emulsion of the strains recovered from the fecal material. Serum was secured from these birds at intervals as noted subsequent to these inoculations and titrated for agglutinins with a stock pullorum antigen. The dosages given and the results obtained are noted in table 1.

TABLE 1.—*Serological tests of three Single-Comb Rhode Island Red pullets after inoculation with Salmonella pullorum secured from bird no. 1862*

Pullet no.	Dosage	Serological tests indicated periods after inoculation		
		11 days [a]	18 days	48 days
	Cc			
1326	1	Positive 1:50	Positive 1:2,000	Positive 1:1,000.
1388	2	Positive 1:50	Positive 1:300	Positive 1:200.
1315	3	Positive 1:50	Positive 1:2,000	Positive 1:1,000.

[a] Dilutions run 11 days after inoculation were in 1:25 and 1:50 strength only.

Subsequent to inoculation these birds laid 43 eggs, and *Salmonella pullorum* was recovered from 13 of these—2 from bird 1315, 6 from bird 1326, and 5 from bird 1388. Inoculation studies with these strains were likewise made with six Rhode Island Red chicks at 3 days of age. These were given graduated doses of the same strains used in the inoculation studies of adults. One chick died of bacteremic pullorum disease. Fifty days after inoculation 3 of the 5 surviving chicks showed agglutinins against a pullorum-disease antigen in a 1 : 25 dilution.

The strain isolated from bird 1631 was inoculated into 2 Single-Comb Rhode Island Red pullets and 3 hens of the same breed. These birds had given negative reactions for pullorum disease in three successive tests conducted at intervals of 30 days. They received inoculations per os of a 24-hour bacillary saline emulsion of the strain isolated from bird 1631. Serum was secured from these birds at intervals as noted subsequent to these inoculations and titrated for agglutinins with a stock pullorum antigen. The dosages given and results secured are shown in table 2.

TABLE 2.—*Serological tests of 2 Single-Comb Rhode Island Red pullets and 3 hens of the same breed after inoculation with Salmonella pullorum secured from bird 1631*

Bird no.	Dosage	Serological tests indicated periods after inoculation		
		8 days	14 days	140 days
	Cc			
718	3	Positive 1:25	Doubtful 1:25	Negative.
1106	3	Negative	Negative	Do.
1143	1	do	do	Do.
1392	3	do	do	Doubtful 1:25.
1361	2	do	Positive 1:25	Positive 1:25.

Subsequent to inoculation the birds laid 34 eggs, and *Salmonella pullorum* was obtained from 1 egg laid by bird 1361. Chick inoculations from the strains used failed to produce demonstrable agglutinins against an antigen of *S. pullorum*.

OBSERVATIONS ON THE PRESENCE OF THE BACTERIOPHAGE

During the course of this experiment four controls gave negative results, but a fifth yielded several small colonies, which were transferred to indicator plates. After incubating for 24 hours these colonies presented a moth-eaten appearance. After 48 hours they were almost completely lysed and could not be transferred. This indicated that a bacteriophage was active in some of the fecal dilutions, and negative cultures from all birds were accordingly cross-streaked with a broth culture of *Salmonella pullorum*, lysis being evident where the new and old streaks crossed when the phagic principle was sufficiently strong. In this manner the lytic agent was detected on one or more plates from 5 of the last 13 birds studied.

SUMMARY

An organism with the true morphological, cultural, and biochemical characteristics of *Salmonella pullorum* was isolated from the feces of 2 of 21 known adult carriers of pullorum disease.

The isolations recovered were obtained from one bird of proven constant reaction to the test for pullorum disease, and from one bird of proven intermittent reaction.

The organisms isolated from the feces of these birds produced the disease when administered per os to proven negative adult birds, and one strain produced the disease in 3-day-old chicks when administered in the same way.

Lytic factors that were sufficiently strong to prevent the recovery of *Salmonella pullorum* from the fecal samples were repeatedly encountered.

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